

Guidelines for retention, storage, and use of residual blood spot samples after newborn screening analysis: Statement of the Council of Regional Networks for Genetic Services.

Therrell et al., Biochem Mol Med 1996; 57:116-124

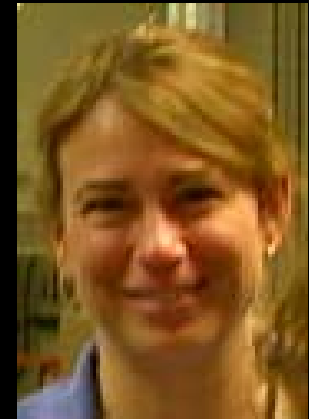
- “..An extensive research of the literature concerning stability of analytes in DBSs was performed, but was of minimal value in making decisions about long-term storage.”
- “***..The general conclusion from these published studies was that data are not available for predicting stability outcomes from long-term storage of DBSs...***”

USE OF RESIDUAL BLOOD SPOTS

- Newborn screening programs store residual blood spots for 3 main reasons:
 - Quality control
 - Research
 - Post-mortem/forensic studies
- The value of residual specimens for NBS purposes is contingent on the stability of analytes and enzymes over time.
- It is important to define the optimal storage conditions.

STABILITY STUDY

The aim of this project is to evaluate the stability of the analytes/enzymes as measured by tandem mass spectrometry (MS/MS) and by traditional methods of screening for biotinidase deficiency, congenital adrenal hyperplasia (CAH), cystic fibrosis, congenital hypothyroidism, and galactosemia over a 2 year period under various environmental conditions.



STABILITY STUDY:

Study design

| | | Room Temperature | | 4 degrees | | -20 degrees | | -70 degrees | |
|--------------------------|----|------------------|------------|-----------|------------|-------------|------------|-------------|------------|
| | | vacuum | desiccated | vacuum | desiccated | vacuum | desiccated | vacuum | desiccated |
| <i>Mass Spec Samples</i> | | | | | | | | | |
| Unspiked | M0 | M0 rv | M0 rd | M0 4v | M0 4d | M0 2v | M0 2d | M0 7v | M0 7d |
| Spiked below cutoff | M1 | M1 rv | M1 rd | M1 4v | M1 4d | M1 2v | M1 2d | M1 7v | M1 7d |
| Spiked at cutoff | M2 | M2 rv | M2 rd | M2 4v | M2 4d | M2 2v | M2 2d | M2 7v | M2 7d |
| Spiked above cutoff | M3 | M3 rv | M3 rd | M3 4v | M3 4d | M3 2v | M3 2d | M3 7v | M3 7d |
| Spiked above cutoff 2 | M4 | M4 rv | M4 rd | M4 4v | M4 4d | M4 2v | M4 2d | M4 7v | M4 7d |
| Spiked above cutoff 3 | M5 | M5 rv | M5 rd | M5 4v | M5 4d | M5 4d | M5 2d | M5 7v | M5 7d |
| <i>CAH samples</i> | | | | | | | | | |
| Spiked above cutoff | H | H rv | H rd | H 4v | H 4d | H 2v | H 2d | H 7v | H 7d |
| Spiked above cutoff 2 | H2 | H2 rv | H2 rd | H2 4v | H2 4d | H2 2v | H2 2d | H2 7v | H2 7d |
| <i>TSH samples</i> | | | | | | | | | |
| Spiked above cutoff | S | S rv | S rd | S 4v | S 4d | S 2v | S 2d | S 7v | S 7d |
| Spiked above cutoff 2 | S2 | S2 rv | S2 rd | S2 4v | S2 rd | S2 2v | S2 2d | S2 7v | S2 7d |
| <i>CF samples</i> | | | | | | | | | |
| Spiked above cutoff | F | F rv | F rd | F 4v | F 4d | F 2v | F 2d | F 7v | F 7d |
| Spiked above cutoff 2 | F2 | F2 rv | F2 rd | F2 4v | F2 4d | F2 2v | Fs 2d | F2 7v | F2 7d |
| <i>Biotinidase/GALT</i> | | | | | | | | | |
| Unspiked | B | B rv | B rd | B 4v | B rd | B 2v | B 2d | B 7v | B 7d |

STABILITY STUDY: Schedule of testing

| | |
|-----------|--|
| TIME 0 | run 13 samples for To, then store at room temp for 3 days to mimic mailing |
| 3 DAYS | run 13 samples for To' then place samples at 10 storage conditions |
| 7 DAYS | run 13 x 10 storage conditions |
| 14 DAYS | run 13 x 10 storage conditions |
| 1 MONTH | run 13 x 10 storage conditions |
| 2 MONTHS | run 13 x 10 storage conditions |
| 3 MONTHS | run 13 x 10 storage conditions |
| 4 MONTHS | run 13 x 10 storage conditions |
| 5 MONTHS | run 13 x 10 storage conditions |
| 6 MONTHS | run 13 x 10 storage conditions |
| 9 MONTHS | run 13 x 10 storage conditions |
| 12 MONTHS | run 13 x 10 storage conditions |
| 15 MONTHS | run 13 x 10 storage conditions |
| 18 MONTHS | run 13 x 10 storage conditions |
| 24 MONTHS | run 13 x 10 storage conditions |

Each testing event

| | |
|--------------|---|
| Mass Spec | 30 min elution 6 samples x 10 storage conditions, in duplicate = 120 wells 90 min elution 6 samples x 10 storage conditions, in duplicate = 120 wells 240 wells total |
| CAH, TSH, CF | 2 samples x 10 storage conditions, in duplicate = 40 wells per assay |
| BIO, GALT | 1 sample x 10 storage conditions, in duplicate = 20 wells per assay |

Stability of Analytes and Enzymes in Stored Blood Spots.



Filter paper prepared for this project (2,940 blood spots)

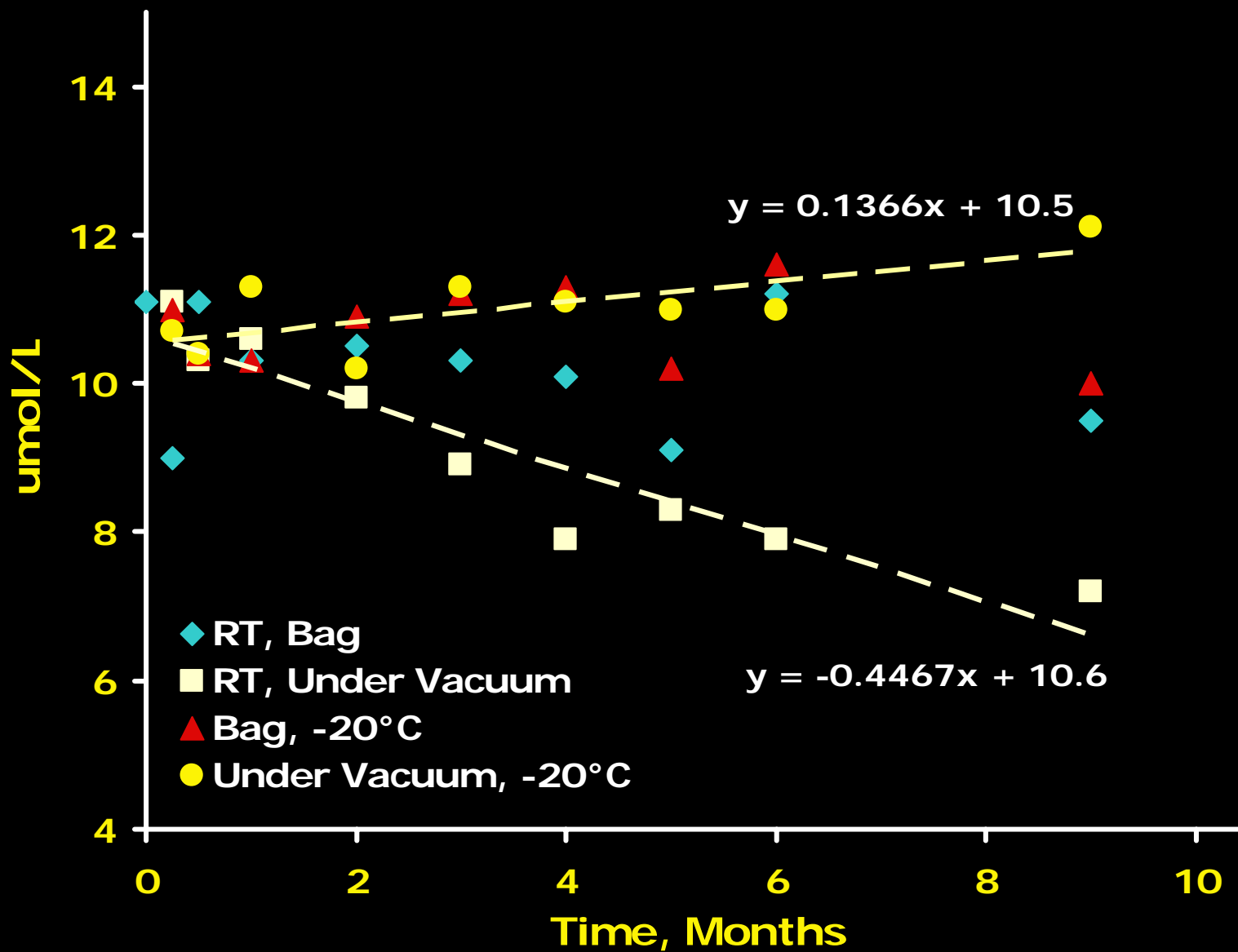
Statistical analysis

- **60 different combinations for the analysis**
 - **Storage (2 conditions): bag + desiccant or vacuum**
 - **6 Levels of concentrations**
 - **Temperature (5 conditions): RT, 4°C, -20°C separated, -20°C not separated, -70°C**

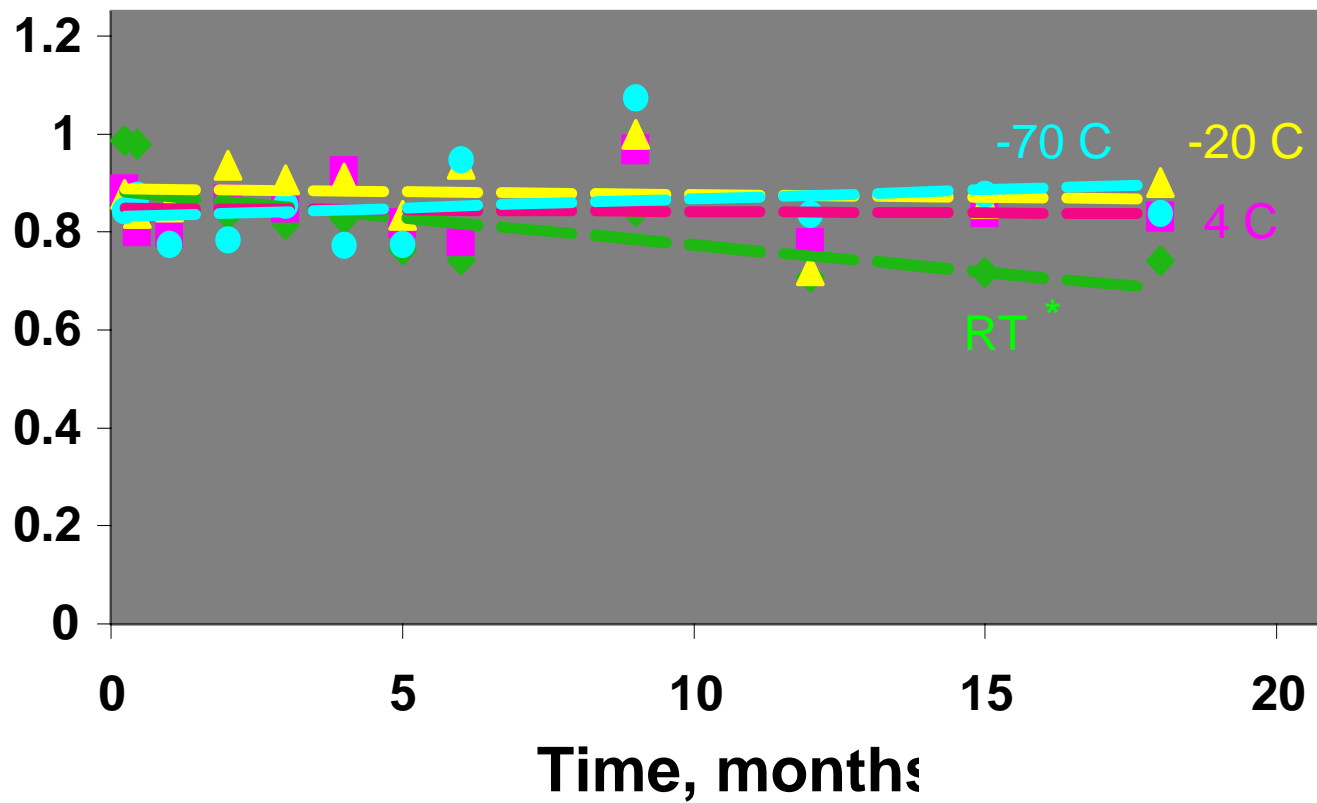
Statistical analysis

- **For each analyte :**
 - **Calculate stability coefficient at every condition at each of the 60 possible combinations.**
 - **Stability coefficient calculated using ANOVA, to see least amount of variation during the 9 months**
 - **Rank the conditions from most stable to least stable using response surface modeling (statistical method that looks at the various conditions as a smooth surface to calculate the conditions that show high or low yield values, analysis method to find the best and the worst condition).**
- **Optimize for all analytes to find the best conditions.**

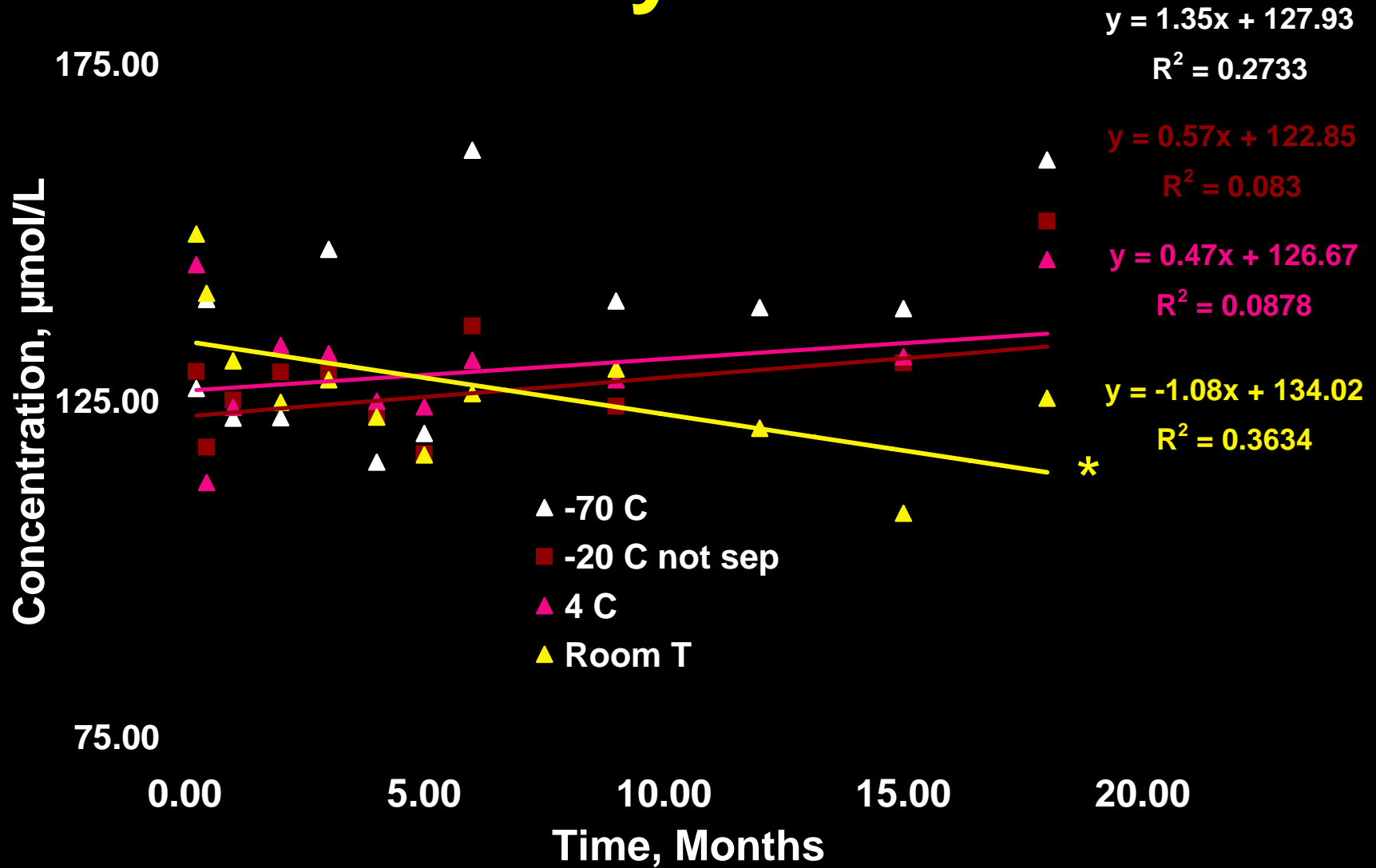
C3-carnitine



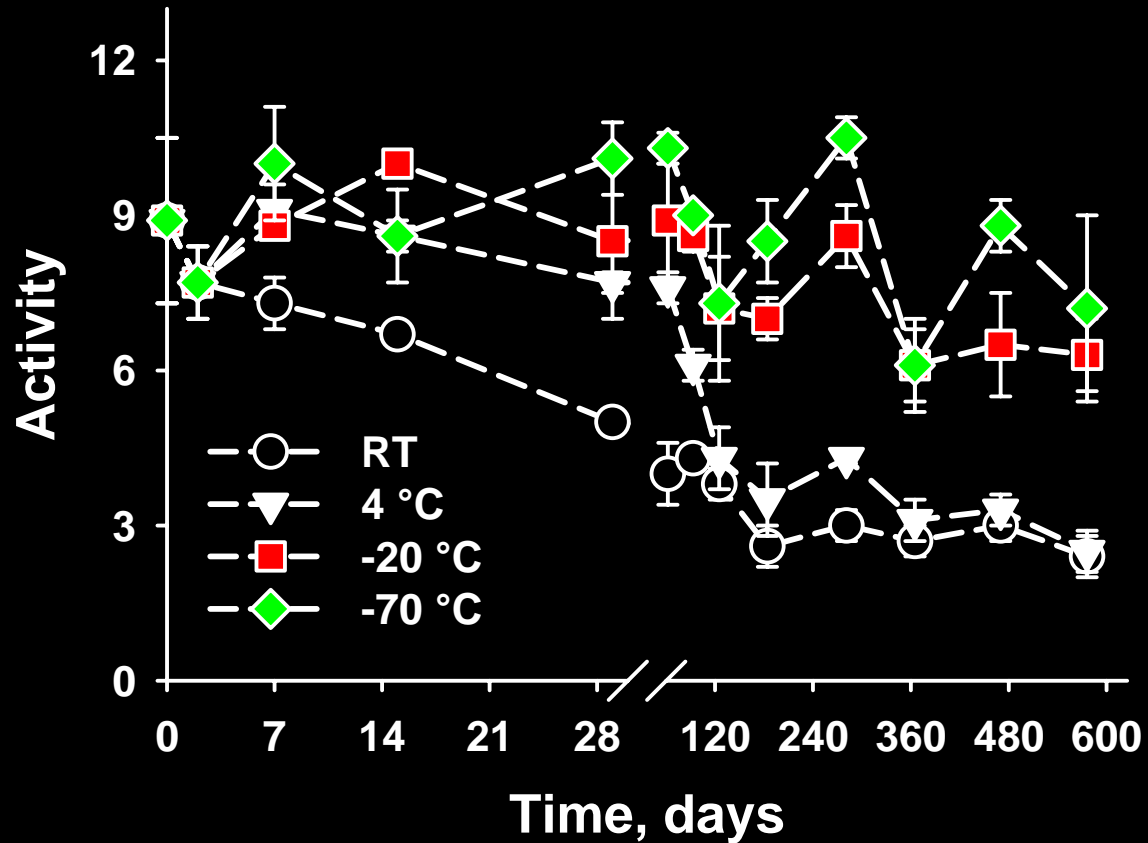
C16-carnitine



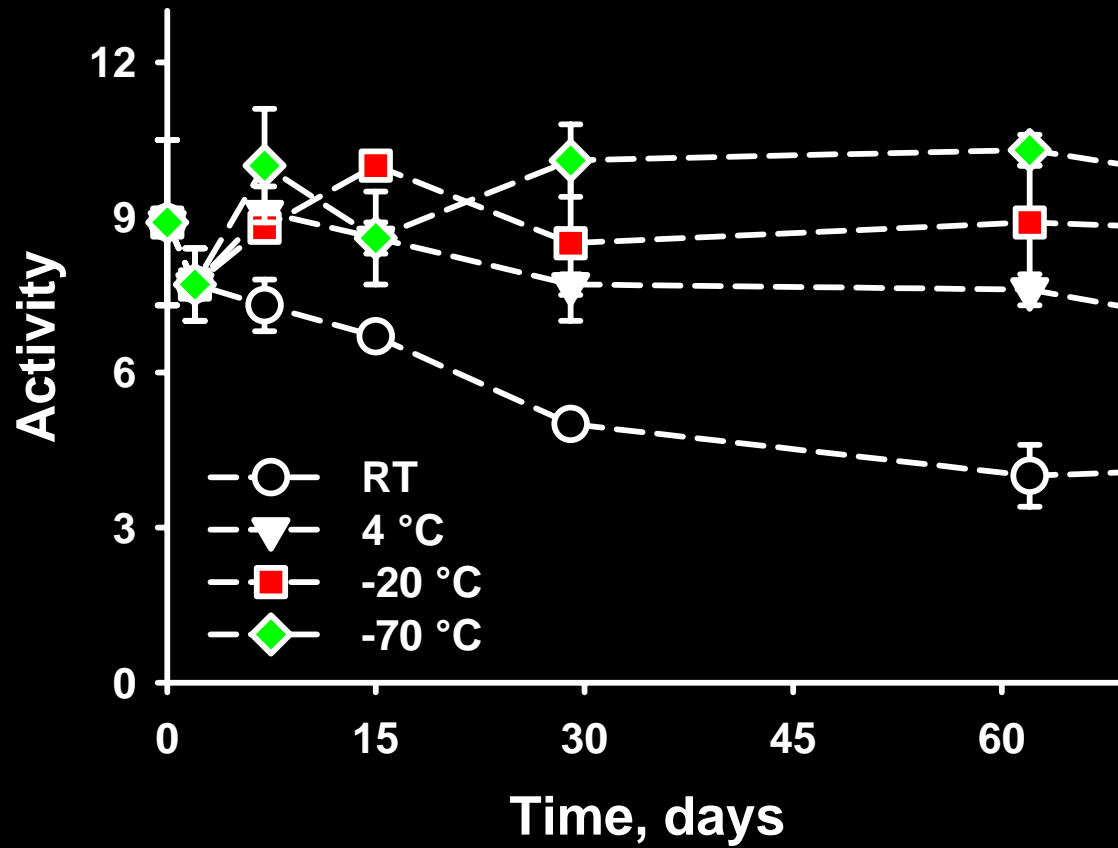
Phenylalanine



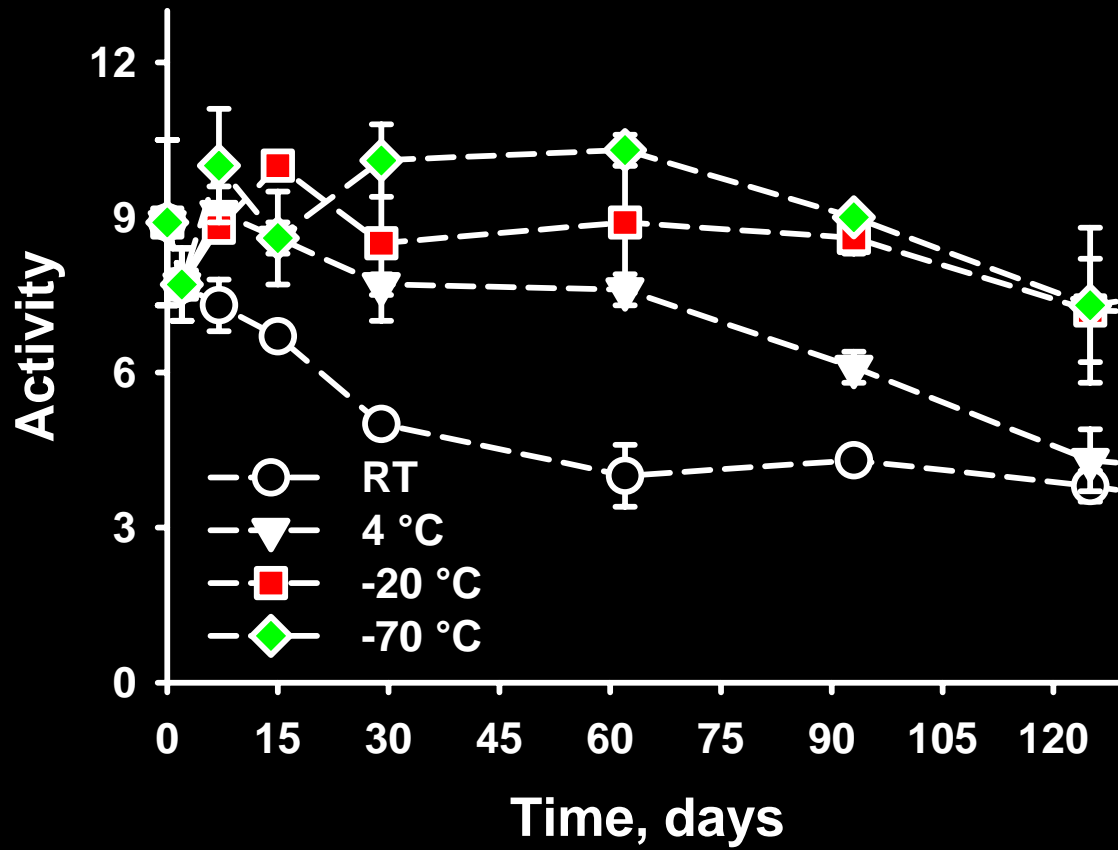
Galactose-1-phosphate uridylyltransferase (GALT)



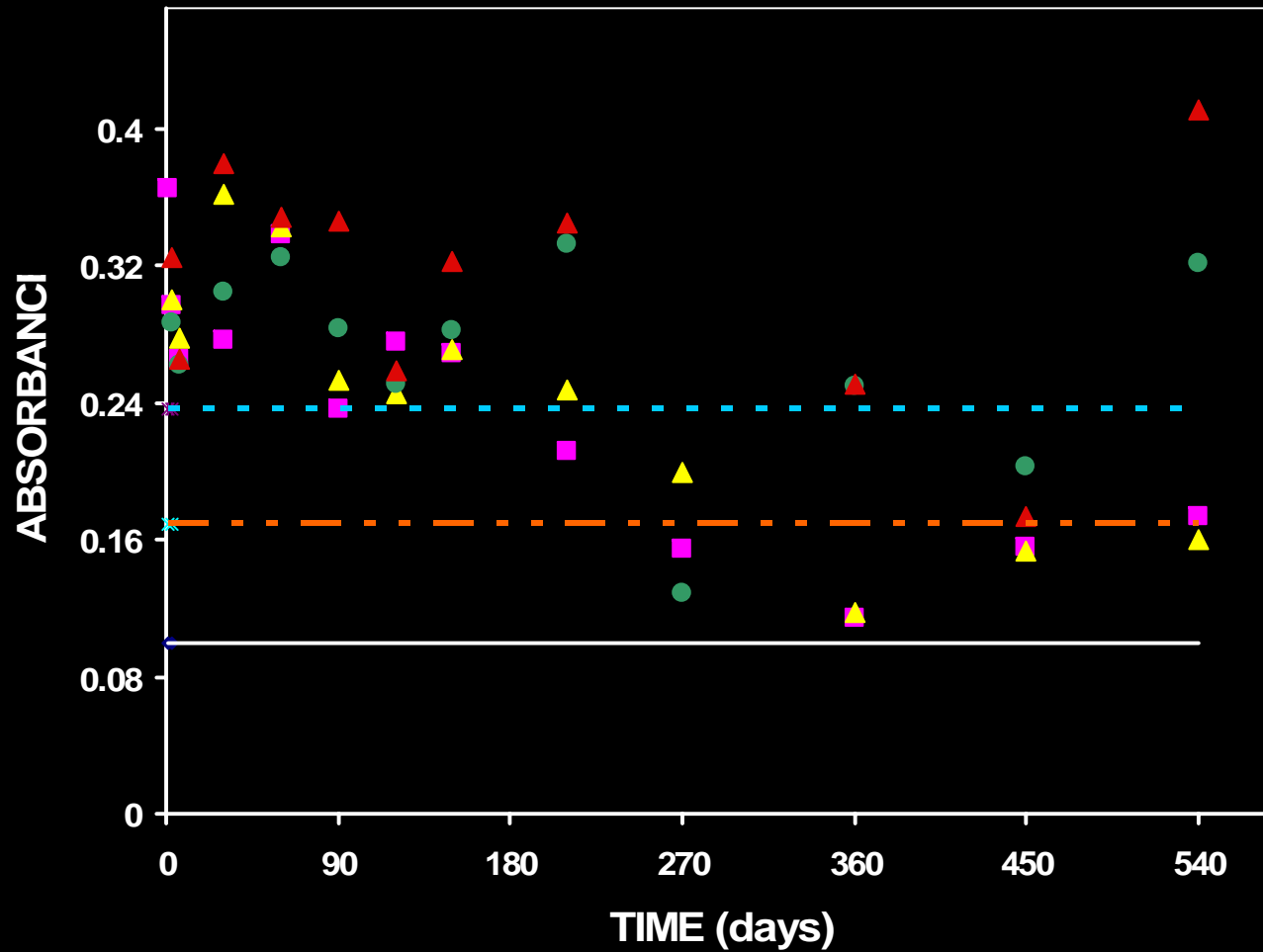
GALT



GALT



Biotinidase



■ 'RT' ▲ 'C 4' ● 'C 20-' ▲ 'C 70-'

Summary

- **Evaluation of the long-term stability of analytes and enzymes in different storage conditions will determine the appropriate storage conditions and use of residual blood spots.**

THANK YOU!



ARUP NBS Laboratory

THANK YOU!

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