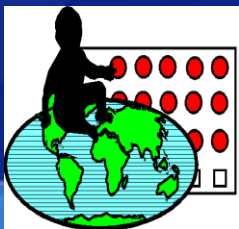


Short and Long Term Stability of 3-Hydroxy acylcarnitines Enriched Dried Blood Spots Stored at Various Temperatures and Humidities

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MS/MS Analytes Included in NSQAP

Arginine

Citrulline

Leucine

Methionine

Phenylalanine

Tyrosine

Valine

Free carnitine (C0)

Acetylcarnitine (C2)

Propionylcarnitine (C3)

Malonylcarnitine (C3DC)

Isobutyrylcarnitine (C4)

3-Hydroxyisobutyrylcarnitine (C4OH)

Isovalerylcarnitine (C5)

Tiglylcarnitine (C5:1)

Glutaryl carnitine (C5DC)

3-Hydroxyisovalerylcarnitine (C5OH)

Hexanoylcarnitine (C6)

Octanoylcarnitine (C8)

Decanoylcarnitine (C10)

Decenoylcarnitine (C10:1)

Dodecanoylcarnitine (C12)

Myristoylcarnitine (C14)

Tetradecenoylcarnitine (C14:1)

Palmitoylcarnitine (C16)

3-Hydroxypalmitoylcarnitine (C16OH)

Stearoylcarnitine (C18)

Oleylcarnitine (C18:1)

Succinylacetone (SUAC)

17 α -Hydroxyprogesterone

Androstenedione/cortisol/11-deoxycortisol/21-deoxycortisol

ABG, ASM, GAA, GALC, GLA

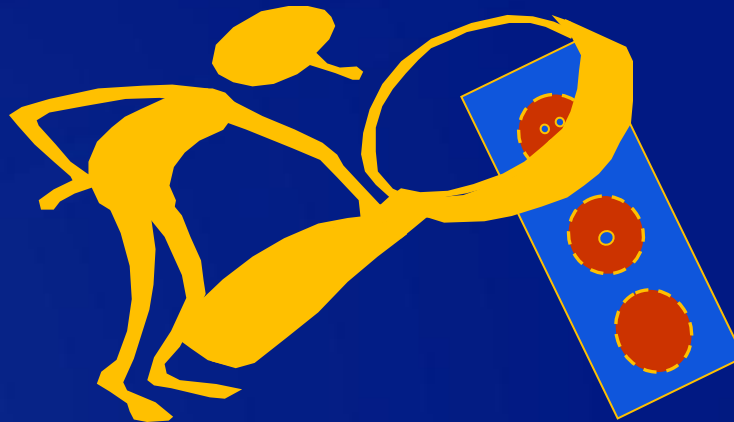
Acylcarnitine enriched Dried Blood Spots

❑ Quality Control Materials – 2 times per year

- 4 levels of enrichment – Base, Low, Intermediate, and High
 - Total of 16 acylcarnitines - C0, C2, C3, C4, C5, C6, C8, C10, C12, C14, C16, C18, C3DC, C5DC, C5OH, C16OH

❑ Proficiency Testing Materials – Quarterly

- DBS enriched with analytes mimicking disease profile
 - Combination of 20 acylcarnitines markers - C0(L), C0(H), C3, C3DC, C4, C4OH, C5, C5:1, C5OH, C5DC, C6, C8, C10, C10:1, C14, C14:1, C16, C16OH, C18, C18:1



Elevation of 3-hydroxy-acylcarnitine

□ C4OH

- Short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) deficiency

□ C5OH

- 3-methylcrotonyl-CoA carboxylase deficiency (3MCC)
- 3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG)
- β -ketothiolase deficiency (BKT)
- Multiple carboxylase deficiency (MCD)
 - including biotinidase deficiency and holocarboxylase synthetase deficiency
- 2- methyl-3-hydroxybutyric acidemia (2M3HBA)
- 3-methylglutaconic aciduria (3MGA)

□ C16OH

- Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency (LCHAD)
- Trifunctional protein deficiency (TFP)

Design of Stability Study for Enriched DBS

DBS enriched with C4OH (3 μ M) and DRS were labeled and placed in Mylar. Identical sets of sample bags were grouped and stored in four different conditions. One bag from each stored condition was collected on designated pull date and re-stored into a freezer set to -70°C until the last sample bags were collected. C4OH and C16OH recoveries were calculated as % of initial concentrations remaining after each storage interval.

1 Month Stability Study for Enriched DBS

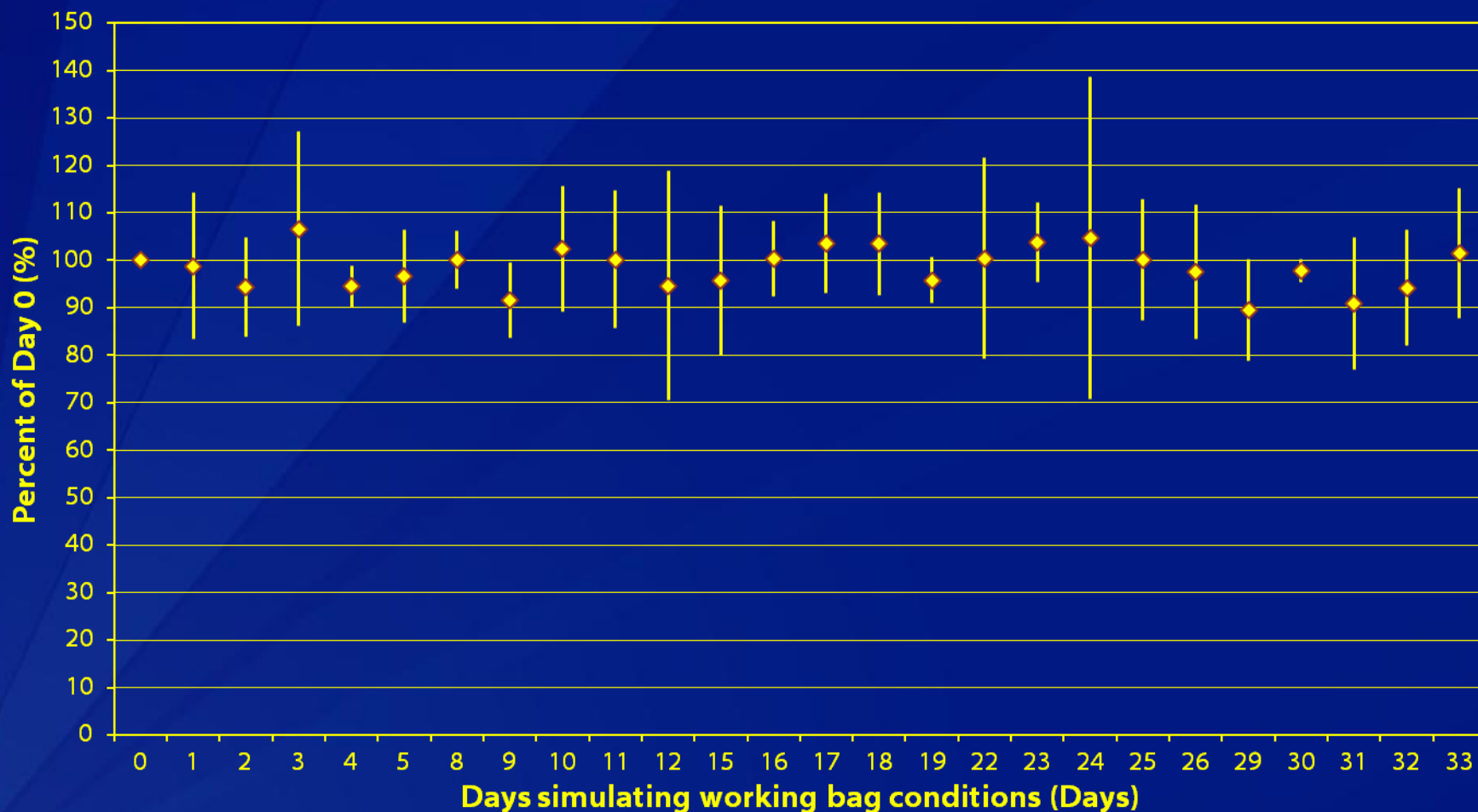
❑ Four different humidity environments:

- Controlled set (Set C): enclosed with desiccant packets
- Opened set (Set O): open to ambient humidity
- Zipped set (Set Z): enclosed with desiccants packets, but kept in a high humidity chamber
- Extreme set (Set E): kept opened in a high humidity chamber for extreme humidity

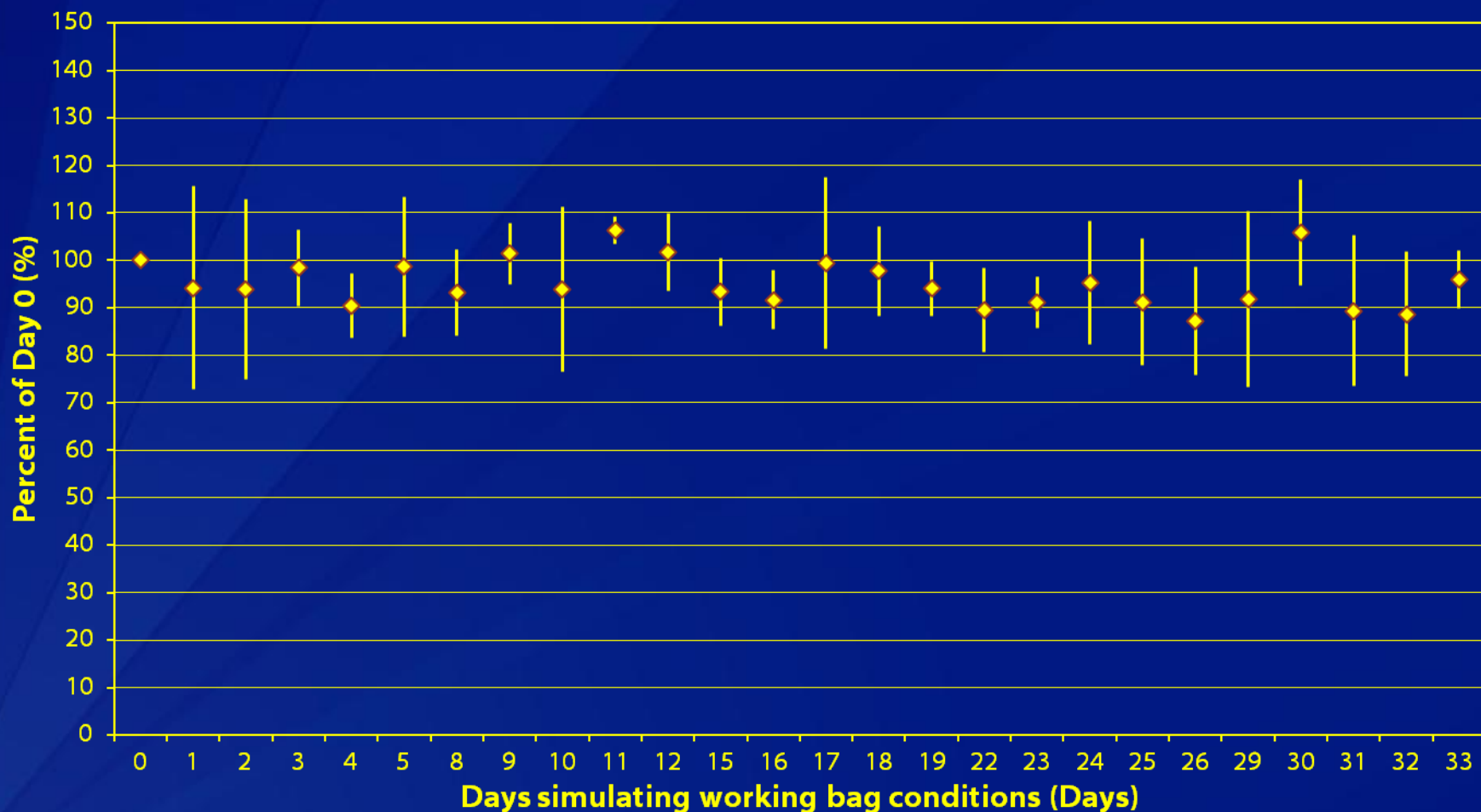
❑ Working Bag Simulation:

- A bag containing all strips of DBS were brought to room temperature to be exposed to ambient humidity and temperature every working day, then brought to 4°C to be stored again
- One sample DBS strip was collected and stored at -70°C each time the bags were opened

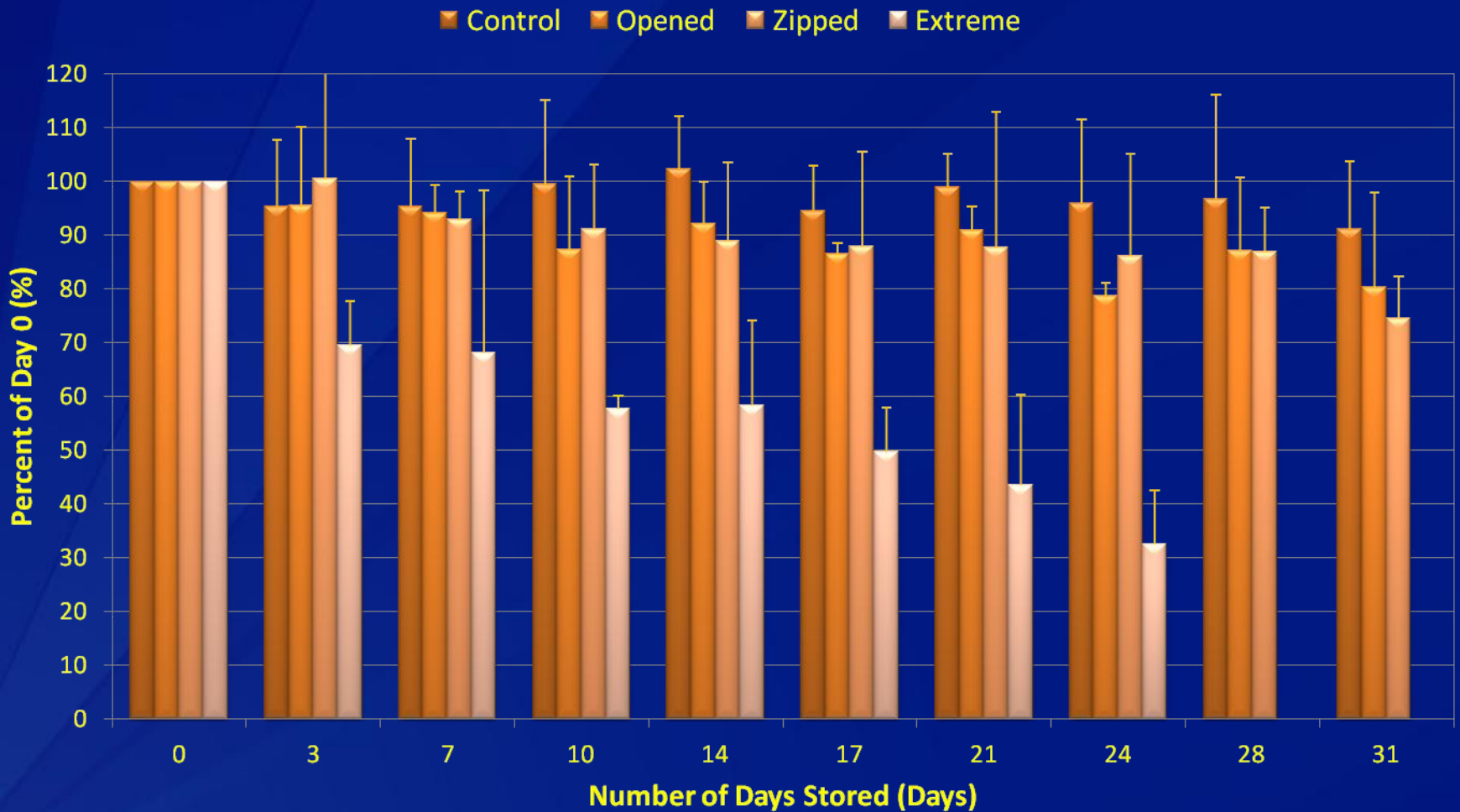
30 Days stability of C4OH enriched Dried Blood Spots stored in a Working Bag (4°C, <30% humidity)



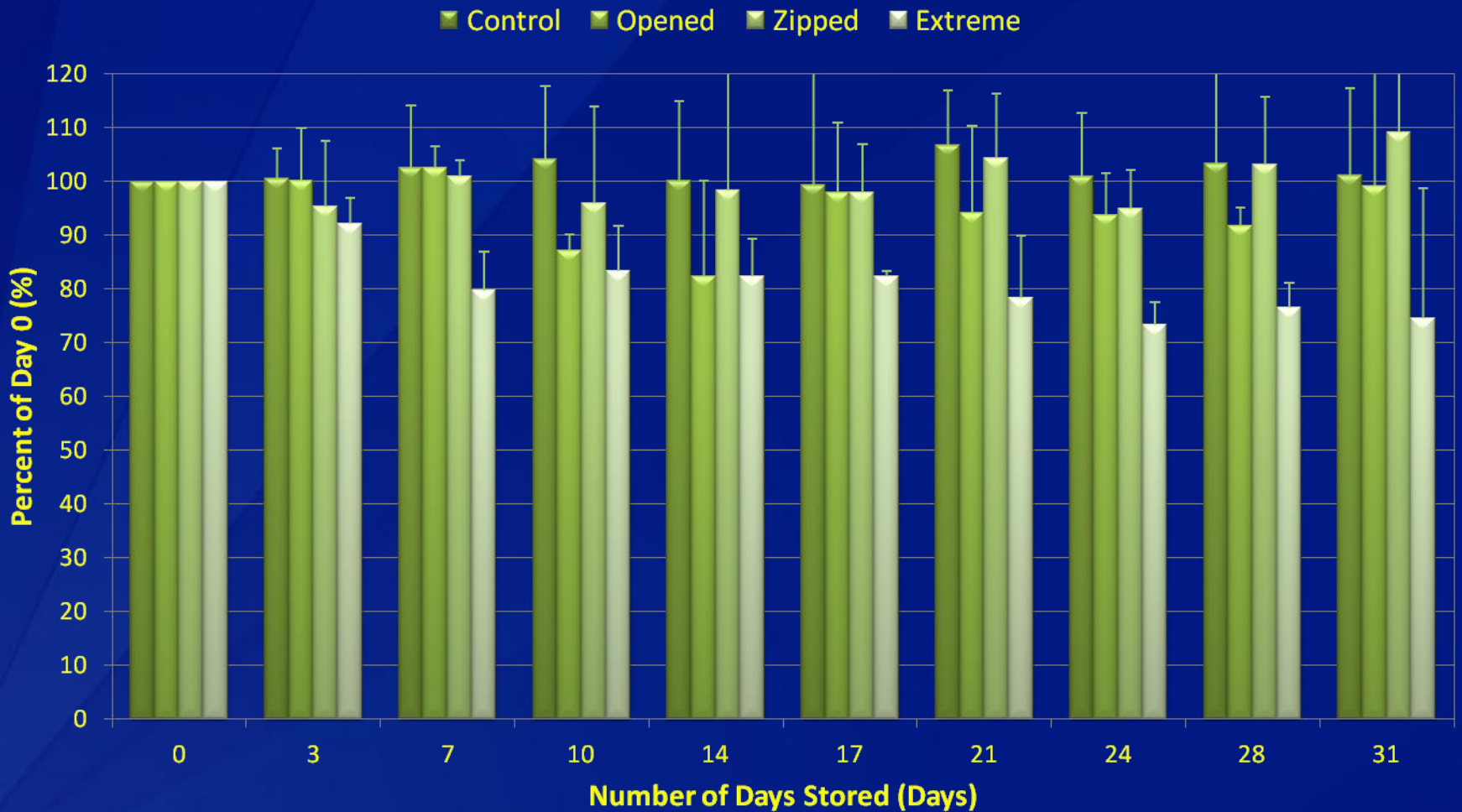
30 Days stability of C16OH enriched Dried Blood Spots stored in a Working Bag (4°C, <30% humidity)



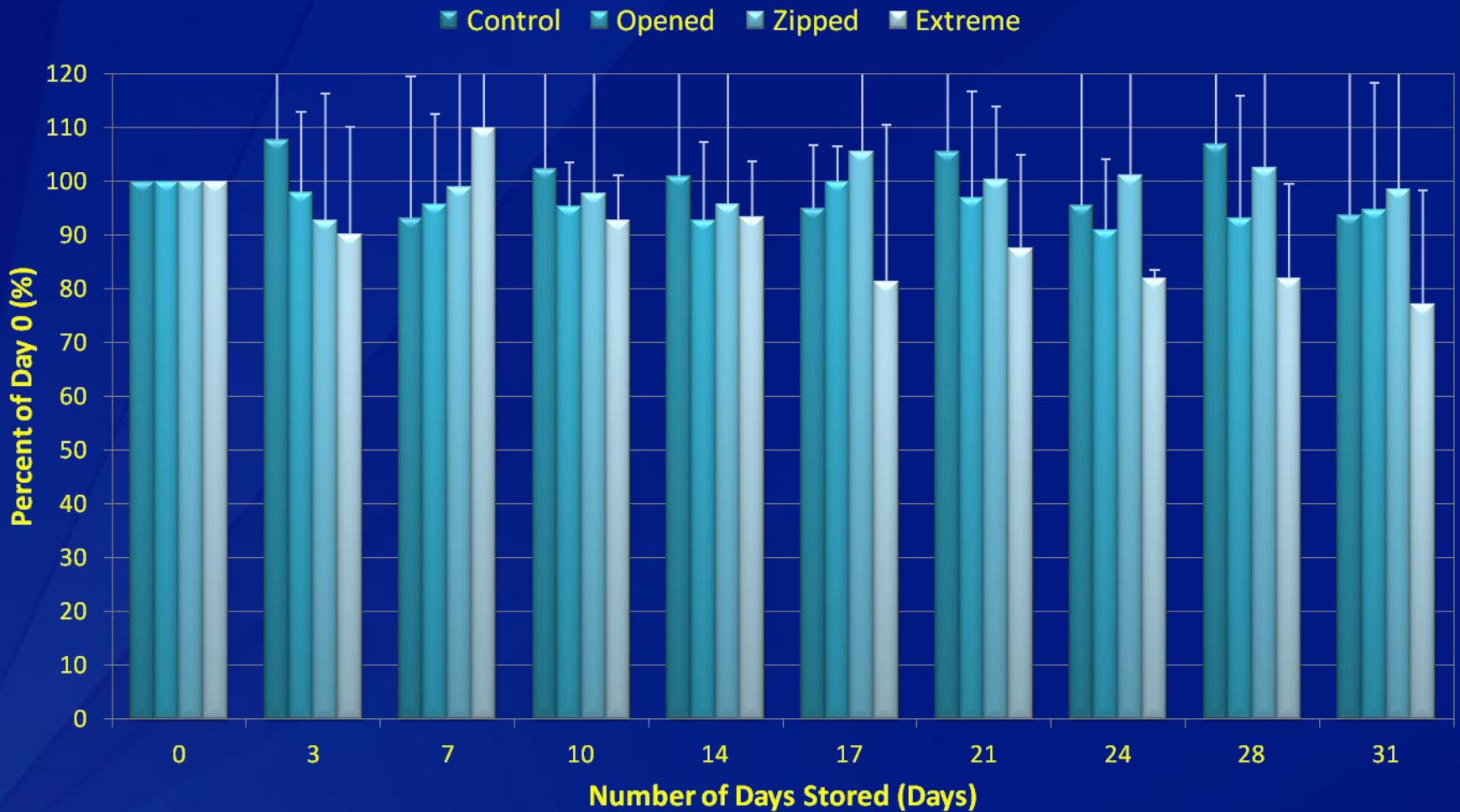
1 Month Stability of C4OH enriched DBS stored at 37°C with different humidities



1 Month Stability of C4OH enriched DBS stored at room temperature with different humidities

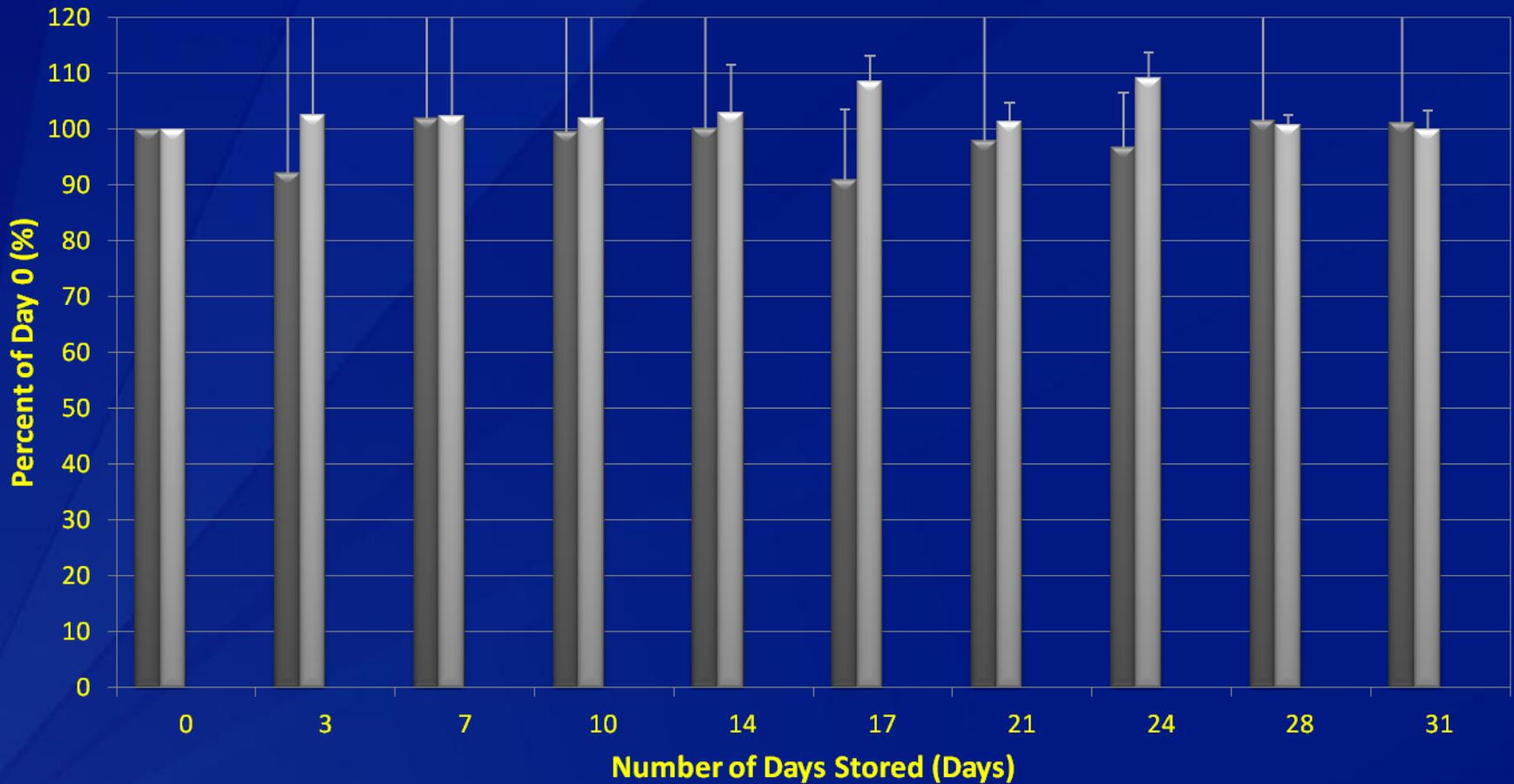


1 Month Stability of C4OH enriched DBS stored at 4°C with different humidities

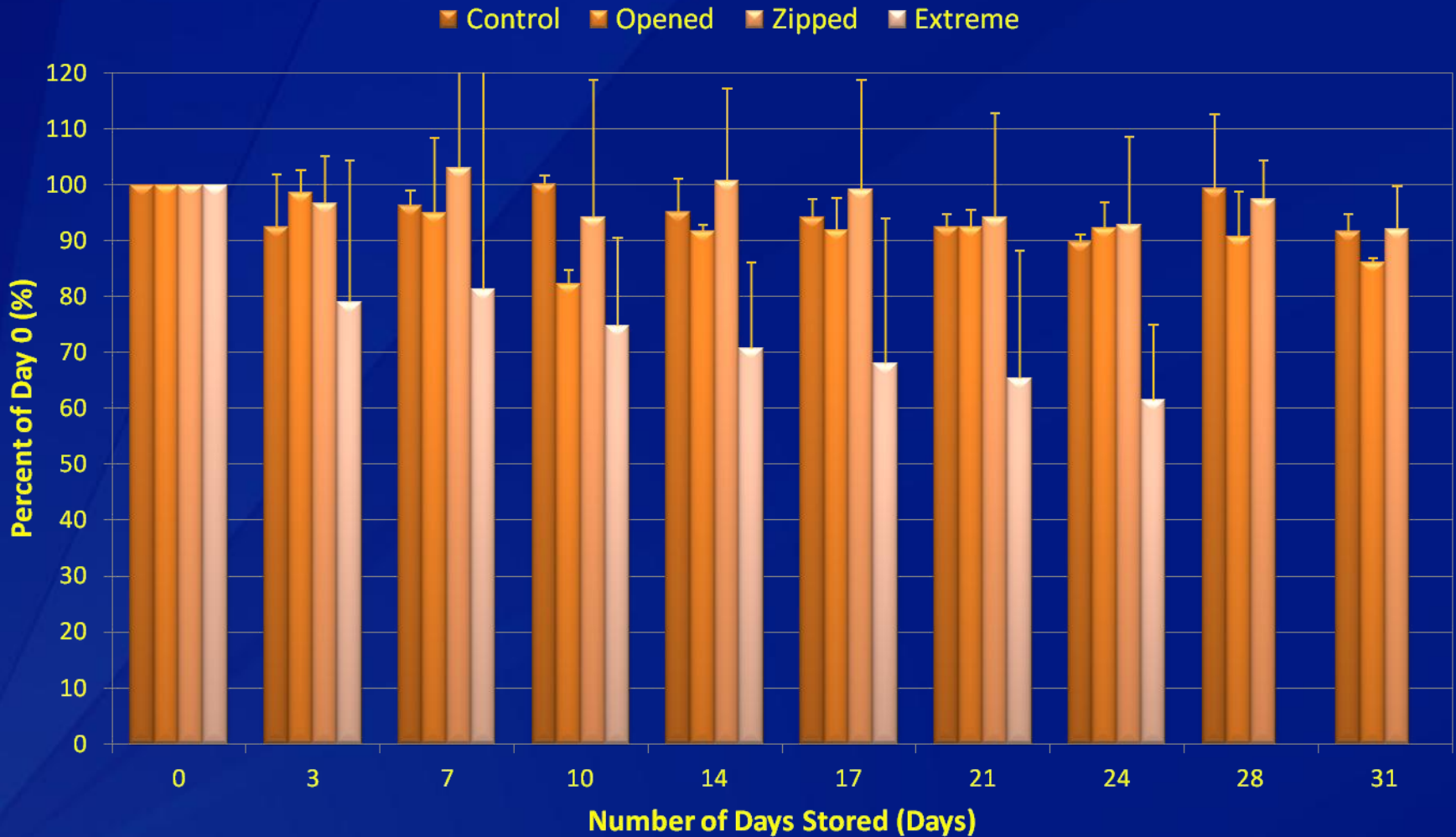


1 Month Stability of C4OH enriched DBS stored at -20°C with different humidities

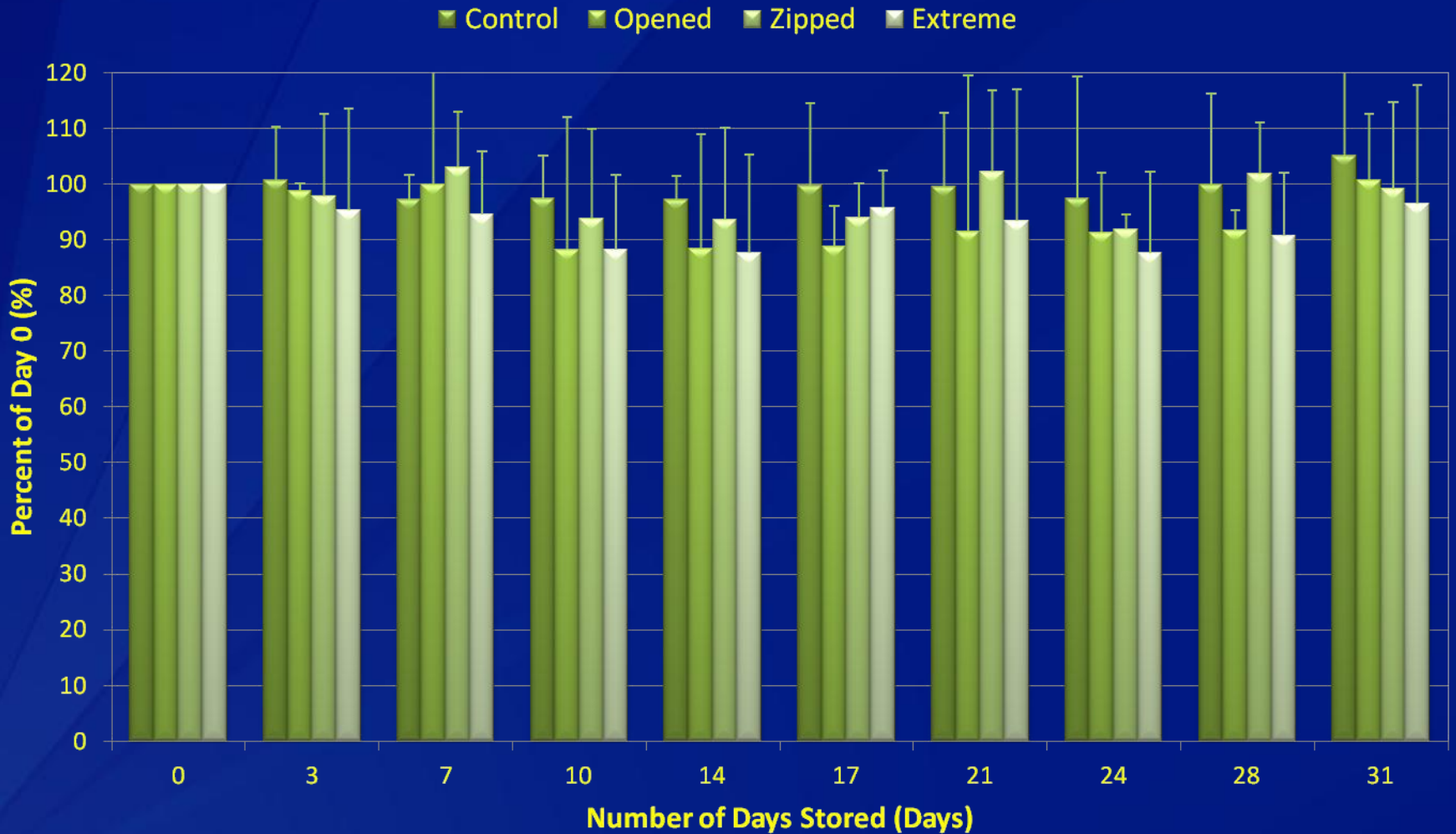
■ Control ■ Opened



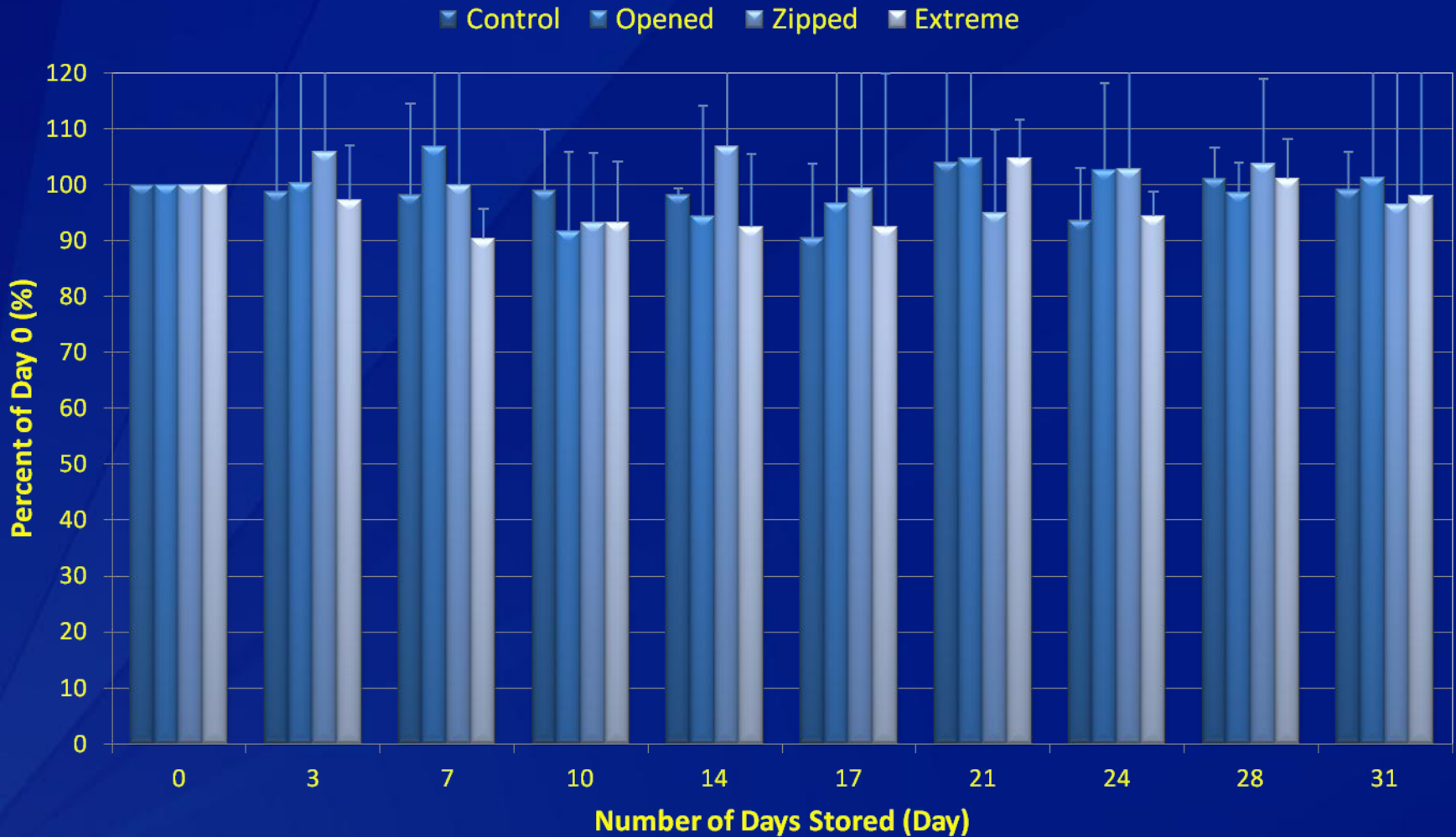
1 Month Stability of C16OH enriched DBS stored at 37°C with different humidities



1 Month Stability of C16OH enriched DBS stored at room temperature with different humidities

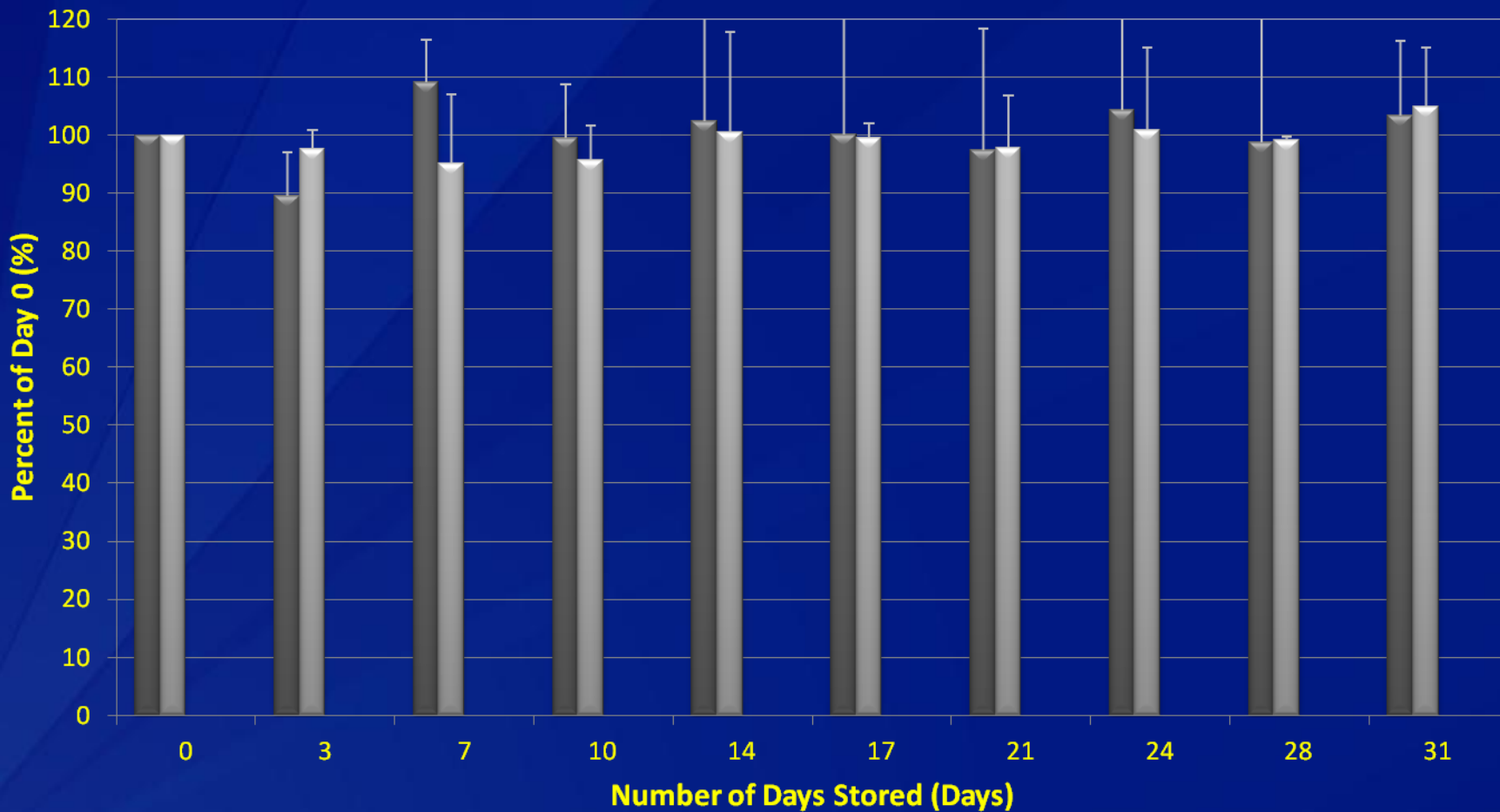


1 Month Stability of C16OH enriched DBS stored at 4°C with different humidities



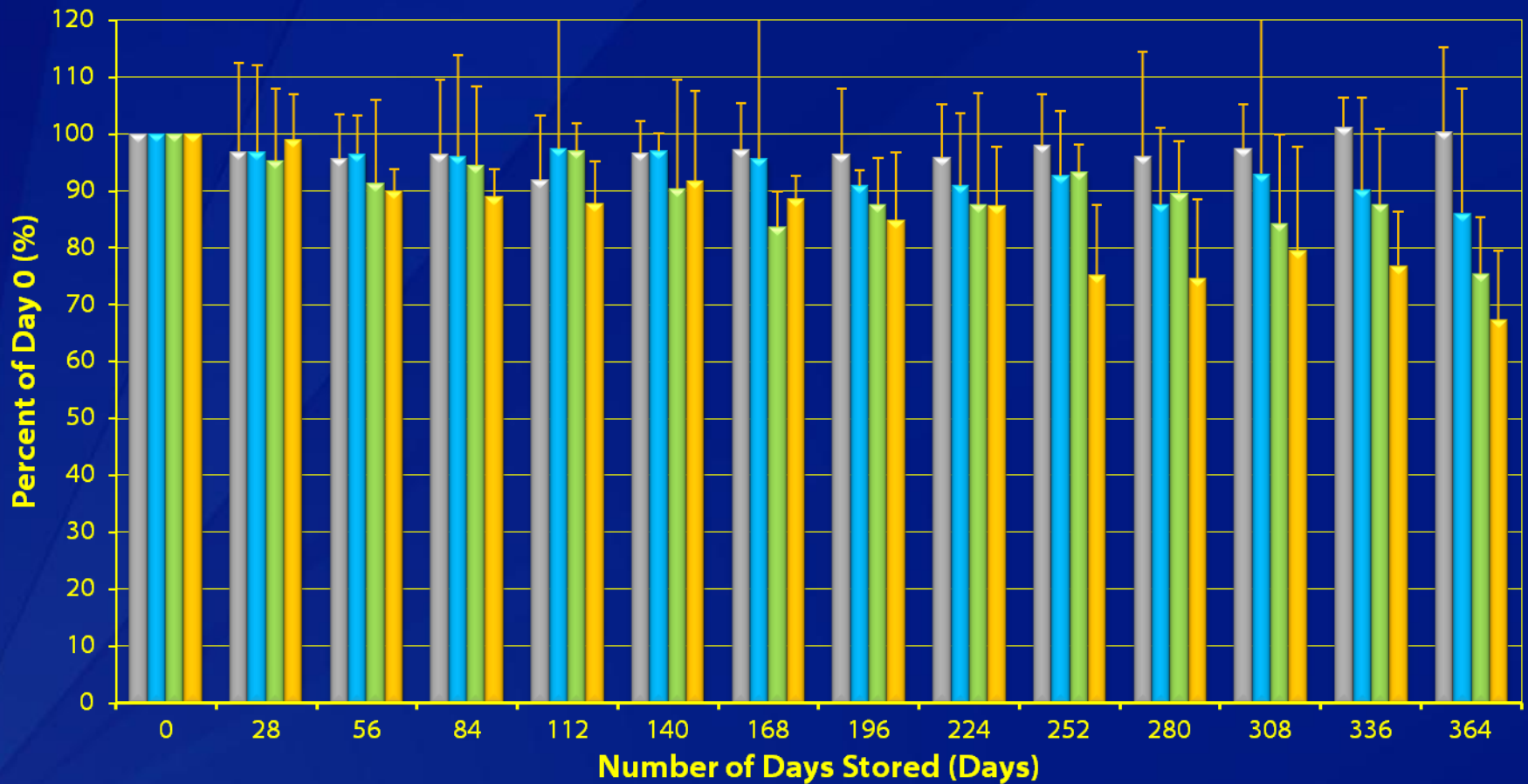
1 Month Stability of C16OH enriched DBS stored at -20°C with different humidities

■ Control ■ Opened



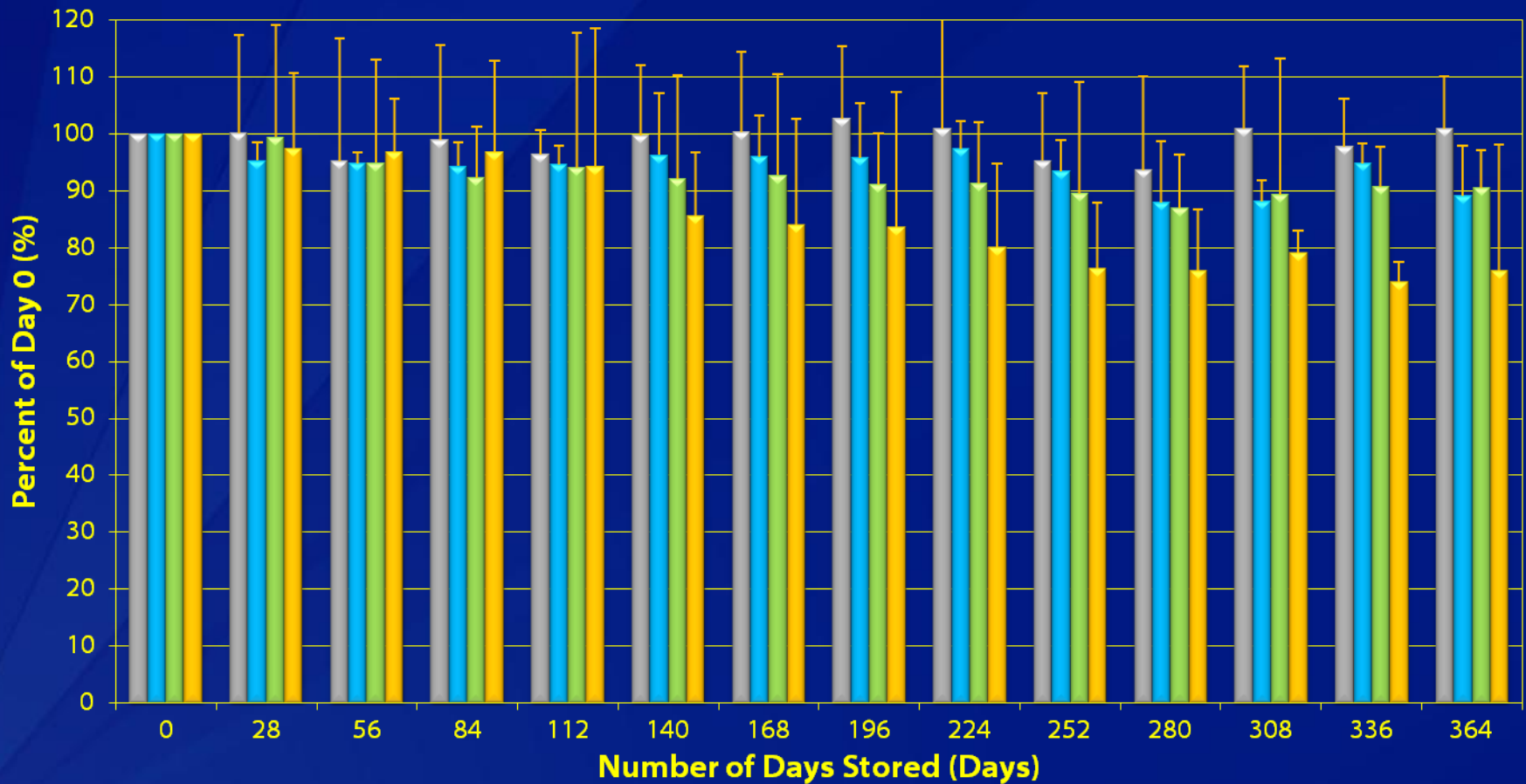
1 Year Stability of C4OH enriched Dried Blood Spots stored at different temperatures with controlled humidity

■ -20°C ■ 4°C ■ R.T. ■ 37°C



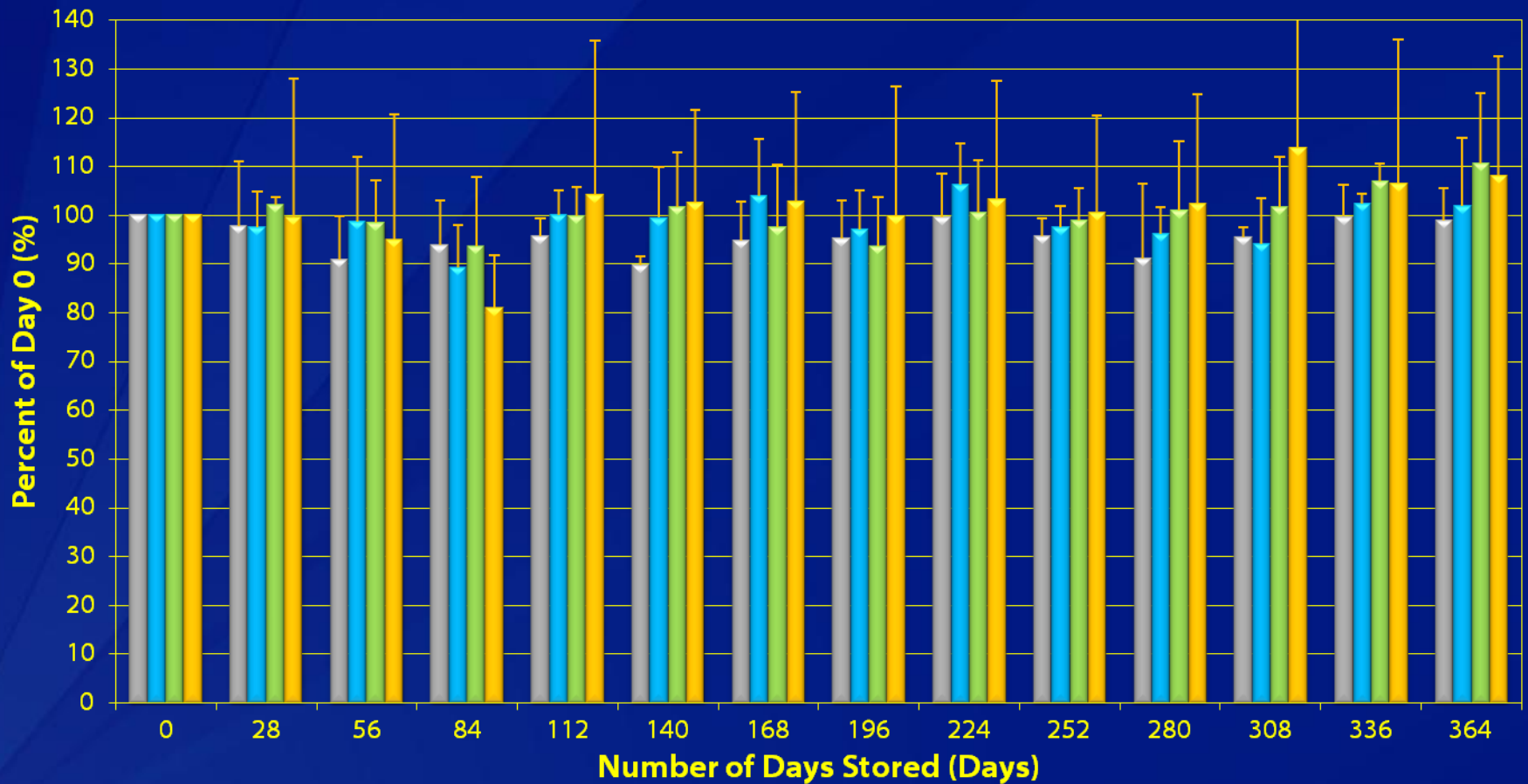
1 Year Stability of C16OH enriched Dried Blood Spots stored at different temperatures with controlled humidity

■ -20°C ■ 4°C ■ R.T. ■ 37°C



Change in free carnitine over 1 year in C4OH and C16OH enriched Dried Blood Spots stored at different temperatures with controlled humidity

■ -20°C ■ 4°C ■ R.T. ■ 37°C



Recovery of 1 Month stability of C4OH enriched DBS stored at different temperatures and humidities

C4OH	-20°C	4°C	R.T.	37°C
Controlled	99	99	100	95
Opened	100	93	93	82
Zipped	-	100	100	80
Extreme	-	79	73	20

Recovery of 1 Month stability of C16OH enriched DBS stored at different temperatures and humidities

C16OH	-20°C	4°C	R.T.	37°C
Controlled	100	99	100	93
Opened	100	100	92	88
Zipped	-	99	98	94
Extreme	-	98	91	52

One year stability of C4OH enriched dried blood spots stored at different temperatures with controlled humidity.

C4OH	-20°C	4°C	R.T.	37°C
1 year (%)	100	88	84	72
6 month (%)	100	94	92	85
t₉₀ (days)	-	304	226	119
t₅₀ (days)	-	1998	1486	780

(Numbers indicate % remained after one year; t₉₀ and t₅₀ indicate estimated time C4OH will decrease to 90 and 50 % of its original concentration.)

One year stability of C16OH enriched dried blood spots stored at different temperatures with controlled humidity.

C16OH	-20°C	4°C	R.T.	37°C
1 year (%)	100	92	90	72
6 month (%)	100	96	95	85
t₉₀ (days)	-	488	379	118
t₅₀ (days)	-	3213	2491	774

(Numbers indicate % remained after one year; t₉₀ and t₅₀ indicate estimated time C4OH will decrease to 90 and 50 % of its original concentration.)

One year stability 3-hydroxy acylcarnitines enriched
dried blood spots stored at different temperatures
with controlled humidity.
(Percent of day zero after one year)

1 year (%)	C3DC*	C4OH	C5OH**	C16OH
37°C	10	72	88	72
R.T.	63	84	89	90
4°C	96	88	99	92
-20°C	98	100	100	100

* T. Lim et al. Stability of malonylcarnitine...; poster presentation at 2008 APHL NBSGTS

**T. Lim et al. Stability of 3-hydroxyisovalerylcarnitine...; poster presentation at 2010 APHL NBSGTS

Summary

- ❑ The samples stored in a working bag simulation did not show any change in recovery of C4OH and C16OH for 1 month
- ❑ The integrity of C4OH and C16OH enriched DBS did not remain intact even in a tightly closed bag with desiccants stored at 37°C for 1 month (80 & 94%, respectively)
- ❑ Enriched C4OH and C16OH in DBS proved to be most stable under -20°C after one year (100%)
- ❑ Recovery of C4OH and C16OH dropped to 88% and 92%, respectively, when stored at 4°C (84% and 90% at R.T.), over 1 year with controlled humidity of <30%

Conclusion

- ❑ The stability of C4OH and C16OH enriched DBS was significantly compromised with high humidity.
- ❑ As we continue to provide PT and QC DBS, we re-emphasize the following based on this study:
 - CDC's 3-hydroxyacylcarnitine enriched DBS are stable at least over 1 year when properly stored (-20°C) with desiccants in a closed bag
 - DBS stored in working bags for daily use need to be stored at 4°C with humidity controls, but no longer than 30 days



Blood spots drying in Nepal

Joanne Mei

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