

Stability of fecal calprotectin extracted with BÜHLMANN CALEX® Cap

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BACKGROUND

Calprotectin, a granulocyte-derived alarmin protein, is an established noninvasive biomarker for intestinal inflammatory status. Fecal calprotectin testing has become the gold standard for diagnostics and management of inflammatory bowel diseases. The aim of this work was to determine the stability of calprotectin in stool extracts prepared with the BÜHLMANN CALEX® Cap stool preparation device.



METHODS

The analyte stability study was carried out using CALEX® Cap extracts of human fecal left-over samples. Extraction was performed for 24 hours at room temperature and samples were aliquoted and frozen at < -20°C.

Five (5) stool samples covering the measuring range of the assay (S1 60; S2 120; S3 250; S4 500; S5 1100 µg/g) were analyzed with the BÜHLMANN fCAL® turbo in four replicates using the cobas® 6000 analyzer.

The baseline calprotectin concentration was measured from thawed aliquots, which were moved to target storage temperatures (2-8°C, 18°C, 28°C, and 37°C) for up to 22 days.

Stability evaluation was performed according to the prior version of CLSI guideline EP25 A1 and the current version (2nd edition) with 10% and 20% acceptance criteria as the allowed deviation from baseline.

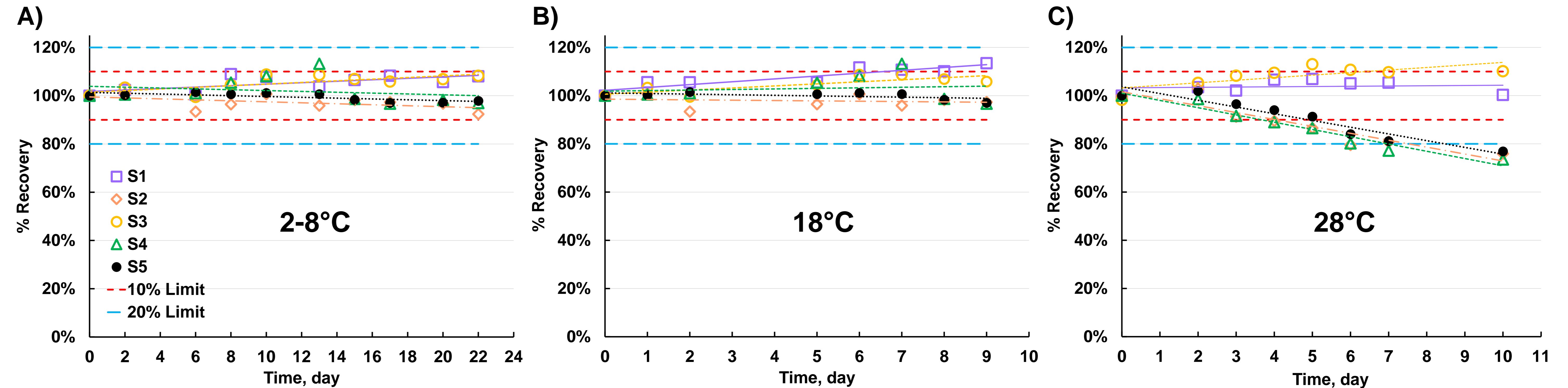


Fig. 1. Overview of changes in % recovery for samples S1 to S5 at A) 2-8°C over a period of 22 days, B) 18°C over a period of 9 days, C) 28°C over a period of 10 days. The 10% and 20% acceptance limits are indicated by dashed red and blue lines, respectively.

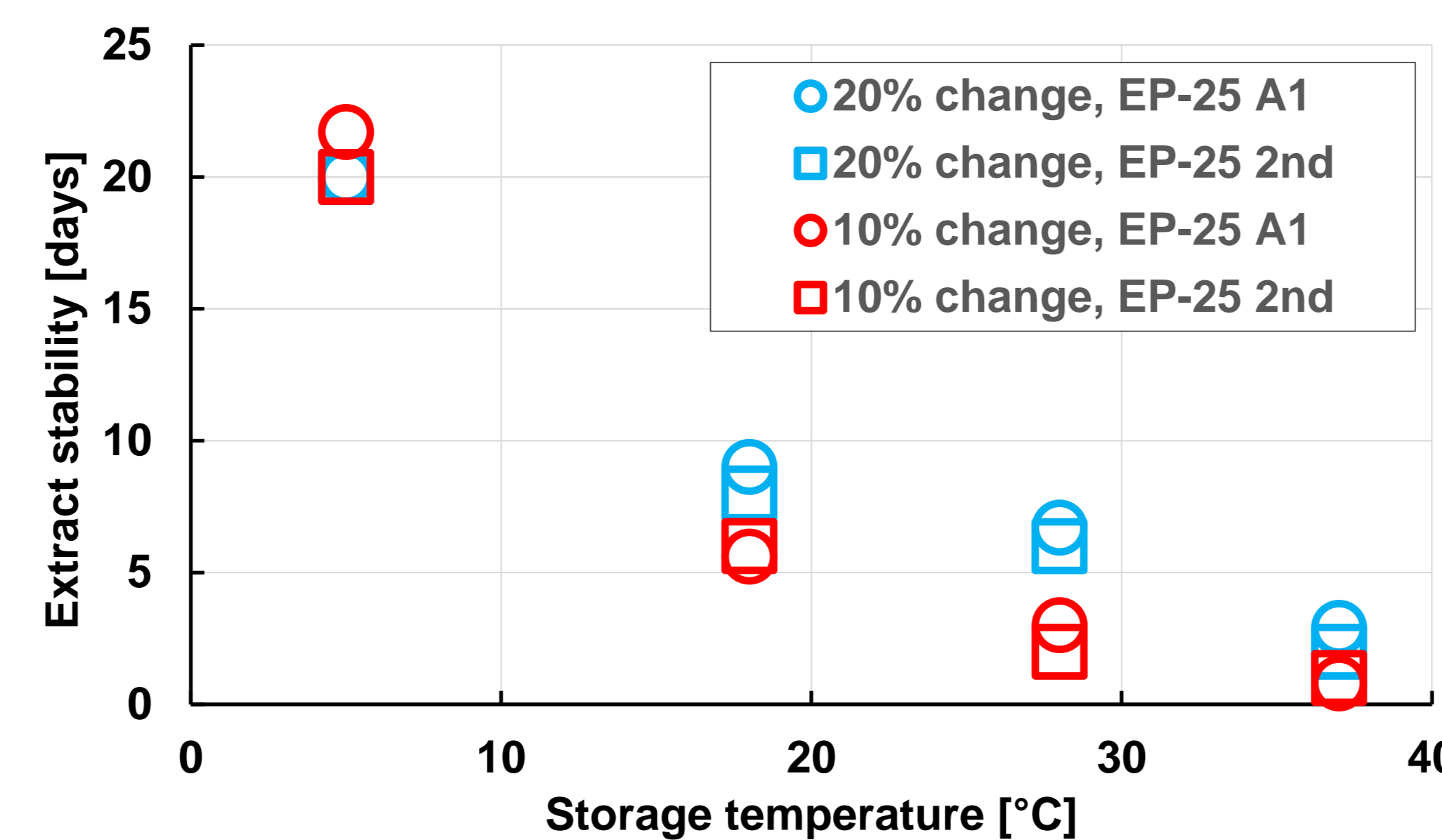


Fig. 2. Comparison of claimed stability durations of CALEX® Cap extracts measured with the BÜHLMANN fCAL® turbo using a 20% and 10% acceptance criteria.

Storage Temperature [°C]	Study Duration [days]	Extract Stability [days]				Extract Stability Claim [days]
		Acceptable change: 20%		Acceptable change: 10%		
		Acc. EP25-A1	Acc. EP25-2nd Ed.	Acc. EP25-A1	Acc. EP25-2nd Ed.	
2-8	22	22	20	20.6	20	15
18	9	9	8	5.6	6	2 (RT)
28	10	6.7	6	3	2	
37	7	2.9	2	0.8	1	N.A.

Table 1. Summary of claimed stability durations for the four (4) different storage temperatures (2 - 8 °C, 18 °C, 28 °C and 37 °C) of CALEX® Cap extracts measured with the BÜHLMANN fCAL® turbo following the EP25 guidelines with a 20% and 10% acceptance criteria.

RESULTS

The extract stability at 2-8°C (**Fig.1 A**) was within the 10% and 20% acceptance criteria for the duration of the study resulting in a stability of 20 days (T_N), which is consistent with a claimed stability of CALEX® Cap extracts at **2-8°C for up to 15 days**.

The extract stability at 18°C (**Fig.1 B**) resulted according to EP25-2nd ed. in a stability of 6 and 8 days for a 10% and 20% acceptance criteria. For storage at 28°C (**Fig.1 C**) extracts are stable for 2 (10% criteria) and 6 days (20% criteria). Fig. 1C nicely shows, that for some samples stability is limited at 28°C while others stay well within ±20% (S2) or even ± 10% (S1) deviation from baseline.

CALEX® Cap extracts can be stored at room temperature (18-28°C) for up to 6 days with an acceptance criteria of 20% or for up to 2 days with an acceptance criteria of 10%. For **storage at 18°C to 28°C the extract stability claim is 2 days**. Results according to EP25-A1 are numerically slightly different but qualitatively identical in our experience. An overview of all claimed extract stabilities is shown in **Table 1**.

CONCLUSION

CALEX® Cap provides a stool preparation method that stabilizes fecal calprotectin concentrations for an increased processing window. This allows significant timely separation between stool collection and the actual measurement of calprotectin.

This specimen stabilization guarantees accurate measurements after sample preparation for 2 days at room temperature and for 15 days when stored at 2-8 °C and thus provides flexibility for diagnostic laboratories.