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Stability of fecal calprotectin using the Diasorin ® extraction kit

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4 1 **Stability of fecal calprotectin using the Diasorin® extraction kit**
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11 3 **Short title: Stability of fecal calprotectin**
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14 19 Number of figure: 1

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21 21 **Keywords:** fecal calprotectin; Diasorin[®]; pre-analytical conditions, stool samples.

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34 24 To the Editor,

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41 26 Fecal calprotectin is a protein used as a biomarker to assist in the diagnosis or
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44 27 rule-out of chronic inflammatory bowel disease (IBD), as well as to evaluate the
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48 28 disease activity or to monitor the effectiveness of medication.[1,2]

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51 29 The Diasorin LIAISON[®]Calprotectin assay is an in vitro diagnostic
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55 30 chemiluminescent immunoassay (CLIA) used for the quantitative measurement of
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58 31 fecal calprotectin present in human stool. The company received the CE marking in
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4 32 2014 and the FDA approval in 2019. Before being loaded on the machine, the
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7 33 specimens must first go through a pre-analytical step that consists of extracting them
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10 34 into a buffer solution using the Liaison® Quantitative Stool Extraction and Test
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14 35 (Q.S.E.T.) Device Plus. Knowledge of the storage conditions for the extracted samples
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17 36 is essential if one wishes to work in series, to defer an assay because of a machine
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21 37 malfunction (if there is no backup option available in the laboratory) or simply to check
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24 38 a result. The manufacturer states that calprotectin is stable in the buffer solution for 7
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28 39 days at -20°C.[3] This short delay can be troublesome in the situations mentioned
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31 40 above.

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35 41 The aim of this study was to evaluate the stability of fecal calprotectin after
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38 42 performing the pre-analytical step under different storage time and temperature
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42 43 conditions. This will allow us to determine if the pre-treated samples can be stored for
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45 44 a period of time longer than that specified by the manufacturer.

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48 45 This non-interventional, monocentric, retrospective study involving data and
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52 46 samples from human participants was carried out in the Grenoble University Hospital
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56 47 according to current French regulation. The investigator (Dr Chovelon, PharmD/PhD)
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59 48 has signed a commitment to comply with Reference Methodology n°004 issued by
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4 49 French Authorities (CNIL). The subjects were all informed and did not oppose; written
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7 50 consent for participation was not required for this study in accordance with the national
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10 51 legislation and the institutional requirements. The raw data supporting the conclusions
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14 52 of this article will be made available by the authors within respect of General Data
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17 53 Protection Regulation, without undue reservation.

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21 54 Patients (n=30) at least 18 years of age, with a prescription for fecal calprotectin
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24 55 analysis as part of their care, were included in the study. Stool samples were
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28 56 introduced into Liaison® Q.S.E.T. Device Plus (ref 319060) pre-filled with extract buffer.
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31 57 The resulting extracted samples were subsequently shaken for 30 min at maximum
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35 58 speed, then analyzed by the Liaison® XS in order to obtain the reference time t_0 .
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38 59 Several aliquots were then prepared; five aliquots were stored at -20°C while five
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42 60 aliquots were stored at 4°C . In a given group, each aliquot corresponded to a delay
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45 61 after which the calprotectin concentration determination was performed, *i.e.* 7, 11, 14,
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49 62 18 or 21 days after t_0 record. Once the analyses were carried out, the samples were
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52 63 destroyed.

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56 64 Twenty-seven of the original thirty patients were considered in the analysis of
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59 65 the results. Three patients were excluded due to the lack of precision on the
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4 66 calprotectin concentration that was lower than 5 mg.kg⁻¹. It should also be noted that
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7 67 an outlier has been removed for a single patient.
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10 68 Our study investigated the influence of storage time and temperature (fixed
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14 69 factors) of samples from different patients (random factor). Violin plots that summarize
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18 70 the full distribution of the data are reported in Figure 1. It was found that the data
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21 71 obtained is more skewed than the Normal distribution. Non-parametric statistical
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25 72 analysis of the mixed model was performed using an Aligned Rank Transform
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28 73 response ANOVA (ART-ANOVA) type III procedure proposed by Wobbrock et al.[4]
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31 74 which is implemented in the ARTool [5] package of R software.[6] This approach is
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35 75 useful when the data is not normally distributed.
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38 76 The independent variables, storage duration and storage temperature, both
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42 77 have a statistically significant impact on the response (p-value = 0.02341 for storage
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45 78 duration and p-value = 6.899e-08 for storage temperature). No statistically significant
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49 79 effect was observed for the interaction duration*temperature level (p-value = 0.9135).
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52 80 This means that the effect of one independent variable (temperature or duration) does
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56 81 not depend on the level of another variable. This statistical test does not allow for the
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4 82 determination of which storage periods yield significant differences in result when
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7 83 compared to the reference time t_0 .
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10 84 To determine whether the calprotectin concentration variations with time and
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14 85 temperature were clinically significant, we first calculated the differences between the
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17 86 median at a given time and temperature and the median at the reference time t_0 . Each
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21 87 median difference was compared to the total change limit (TCL) = $[(2,77 \cdot CVa)^2 +$
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24 88 $(0,5 \cdot CVi)^2]^{1/2}$. [7] In a second step, we calculated the concentration differences for each
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28 89 patient, between data acquired at a specific time and temperature and the reference
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31 90 time t_0 . Each difference was then compared to the reference change value (RCV) =
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35 91 $2,77(CVa^2 + CVi^2)^{1/2}$. [8] The calculation of these indicators considers the within-run
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39 92 analytical imprecision CVa , which was assessed by measuring 30 dilutions of quality
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42 93 control samples prior to this study, and the within-individual biological variability CVi ,
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45 94 which was available in a previous paper. [9]
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49 95 Concerning the clinical viewpoint, as presented in Table 1, the concentration
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52 96 differences for each patient were lower than RCV. However, while the median
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56 97 differences were lower than TCL until day 21 at a storage temperature of -20°C , the
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59 98 one on day 7 at the storage temperature of 4°C turned out to be higher than TCL.
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4 99 According to our findings, the fecal calprotectin concentration is significantly
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7 100 influenced by storage time and temperature. This analyte is not stable at 4°C but
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10 101 remains stable for 18 days at -20°C after the pre-analytical procedure with the Liaison®
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14 102 Q.S.E.T. Device Plus. Compared to previous Diasorin® stability data (7 days at -20°C)
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18 103 [3], our results will allow greater flexibility for laboratories using the Diasorin
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21 104 LIAISON®Calprotectin assay.
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28 106 **Acknowledgments:** Thanks to the company Diasorin® for providing us with the
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31 107 necessary reagents for this study.
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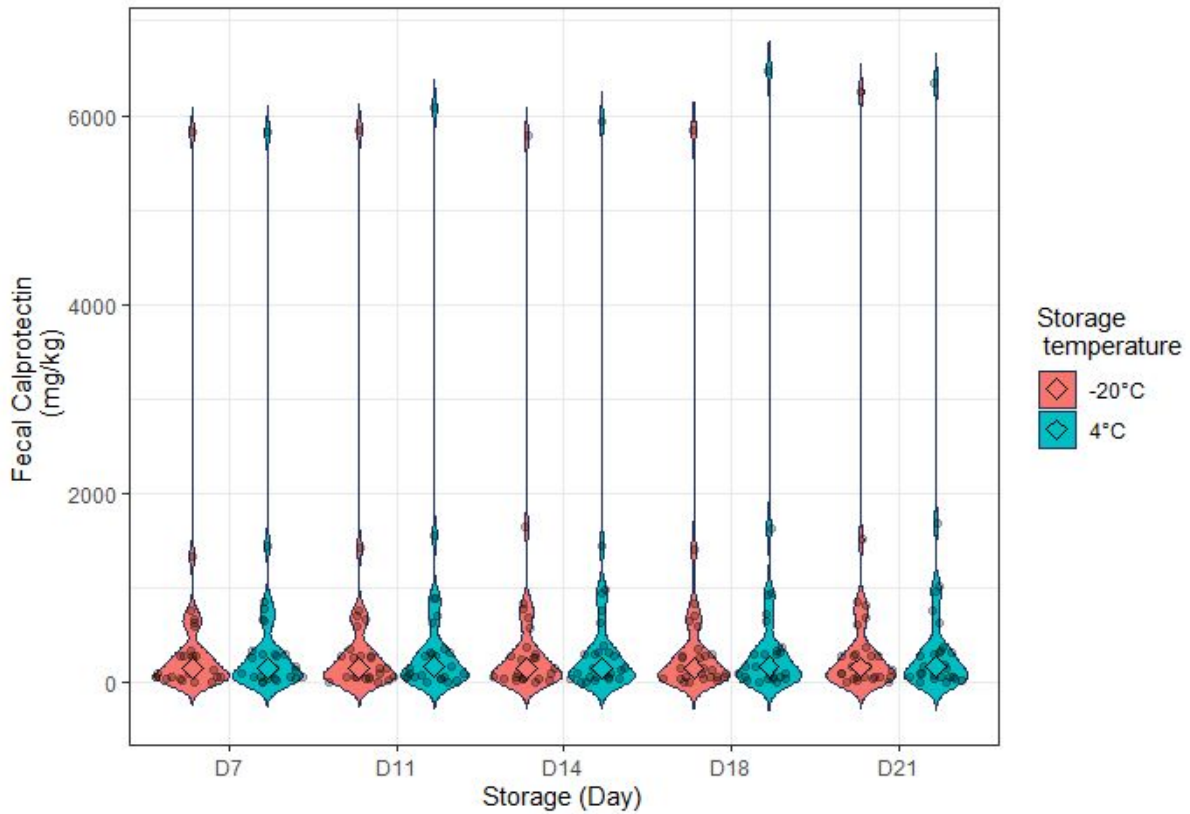
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References

- [1] Ricciuto A, Griffiths AM. Clinical value of fecal calprotectin. *Crit Rev Clin Lab Sci* 2019;56:307–20.
- [2] Walsham NE, Sherwood RA. Fecal calprotectin in inflammatory bowel disease. *Clin Exp Gastroenterol* 2016;9:21–9.
- [3] LIAISON® Calprotectin ([REF] 318960) EN – 54427 - 2021-03). Stillwater : DiaSorin Inc. , MN 55082.USA.
- [4] Wobbrock JO, Findlater L, Gergle D, Higgins JJ. The aligned rank transform for nonparametric factorial analyses using only anova procedures. *Proceedings of the*

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4 133 SIGCHI Conference on Human Factors in Computing Systems, New York, NY,
5
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7 134 USA: Association for Computing Machinery; 2011, p. 143–6.
8
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10
11 135 [5] Kay M, Elkin L, Higgins J, Wobbrock J. ARTool: Aligned Rank Transform for
12
13
14 136 Nonparametric Factorial ANOVAs 2021.
15
16
17 137 [6] R Core Team (2022). R : A language and environment for statistical computing.
18
19
20
21 138 [7] Oddoze C, Lombard E, Portugal H. Stability study of 81 analytes in human whole
22
23
24 139 blood, in serum and in plasma. Clin Biochem 2012;45:464–9.
25
26
27
28 140 [8] Petersen PH, Fraser CG, Lund F, Sölétormos G. Confirmation of analytical
29
30
31 141 performance characteristics required for the reference change value applied in
32
33
34 142 patient monitoring. Scand J Clin Lab 2015;75:628–30.
35
36
37
38 143 [9] Padoan A, D’Inca R, Scapellato ML, Bastiani RD, Caccaro R, Mescoli C, et al.
39
40
41 144 Improving IBD diagnosis and monitoring by understanding preanalytical, analytical
42
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44 145 and biological fecal calprotectin variability. Clin Chem Lab Med 2018;56:1926–35.
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149 **Figure 1.** Violin plots for fecal calprotectin quantification as a function of storage
 150 duration and temperature. \diamond marker represents the median of the data.



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153 **Table 1:** Median differences (lowest value, highest value) in percent, between results

154 at a given time and temperature and results at the reference time t_0 .

	Day 7	Day 11	Day 14	Day 18	Day 21	CVa	CVi	TCL	RCV	Acceptable delay
4°C, %	20.2 (-4.82, +41.5)	24.0 (-1.61, +52.0)	17.8 (-2.56, +57.1)	30.2 (-2.32, +53.9)	35.7 (-10.7, +61.4)	3.10	31.4	17.9	87.4	n/a
-20°C, %	15.5 (-8.46, +22.2)	17.1 (-5.19, +25.5)	10.9 (-8.97, +30.7)	11.6 (-7.69, +21.5)	23.3 (+0.577, +36.3)					18 days

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