Letter to the Editor

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Crioplast® is a reliable device to ensure pre-analytical stability of adrenocorticotrophin (ACTH)

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To the Editor,

Adrenocorticotropic hormone (ACTH) is commonly measured to explore pathologies related to the hypothalamic–pituitary–adrenal axis. However, with a plasma half-life below 20 min, ACTH is well known to be unstable *in-vivo* and *in-vitro* due to proteolytic degradation [1]. For this reason, it is recommended that an EDTA tube be used for blood collection and that the sample be kept in ice water immediately after venipuncture to reduce proteolysis, centrifuged and analyzed within 1 h [2, 3]. Nevertheless, these conditions are difficult to be applied in routine laboratory conditions as it is very common for samples to take more than an hour to reach the laboratory, especially if the patient has been sampled in remote areas.

Transport on ice is also a treacherous way to get the sample to the laboratory as it is difficult to standardize. Furthermore, excessive cooling of the sample can cause hemolysis, which is known to interfere with ACTH measurement [4]. This is mainly due to the release of proteolytic enzymes from red blood cells that destroy small peptides like ACTH, insulin and many others hormones [5].

In recent years, several more convenient transport solutions have been commercialized. Among these, the Crioplast® (LP Italiana, Milano, Italia) device has been adopted by several laboratories in Belgium. This device is a plastic cooling container in the shape of a rectangular parallelepiped, which harbors a recess at the end to accommodate a 100 × 16 mm tube. This device should be placed in the freezer at −20 °C for at least 12 h before use. Since there is currently no data for ACTH stability, we performed the present study to determine whether ACTH is stable over time and whether hemolysis occurs when the sample is stored in the Crioplast®.

For that purpose, one hundred and thirty-five samples were collected from 15 healthy volunteers between 8 and 10 am (9 females and 6 males). All subjects gave informed consent to participate in the study. For each volunteer, nine blood samples were collected in plastic tubes with EDTA-K_2 as anticoagulant (Vacuette® 4 mL EDTA-K_2, Greiner bio-one®, Kremsmünster, Austria). Samples were collected on different days for each volunteer. For each set of nine samples, the first one was centrifuged immediately after collection in a refrigerated centrifuge (4 °C) at 3,500 rpm (≈2,870 *g*) for 10 min and represents the gold-standard condition. For the other samples, four of them were stored at 4 °C for 1, 2, 4 and 8 h before being centrifuged at the same conditions and aliquoted (condition A). Finally, the last four samples were placed in Crioplast® and left at room temperature (22 °C) for 1, 2, 4 and 8 h before being centrifuged and aliquoted (condition B). Following aliquoting, all samples were stored at −80 °C until batch testing on the same analytical run.

All samples were tested using a chemiluminescent immunoassay (CLIA) (LIAISON® ACTH, reference 313221) on a LIAISON® XL analyzer (DiaSorin®, Saluggia, Italy) after appropriate calibration and successful internal control measurement (LIAISON® Control ACTH; reference 319132). After establishing statistical normality, the paired Student's t-test was used to assess the overall difference in mean ACTH concentrations over time between each group tested and the reference group. Differences were considered statistically significant at $p<0.05$. In addition, the average bias as a function of storage time and temperature was calculated in relation to the reference sample and compared with the inter-assay analytical bias announced by the manufacturer.

Our results showed that the mean ACTH concentrations were similar in both conditions. For the reference condition, the mean ACTH concentration (±SD) of the 15 samples was

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