STABILITY OF THE THROMBOXANE METABOLITE 11-DEHYDRO-TXB2AND OF CREATININE IN SAMPLES STORED OVER 5YEARS

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Introduction: Biologic samples or their extracts are often stored for several years in biobanks before assessing specific biomarkers. Therefore, assessing the stability of biologic samples and of specific biomarkers over long-term storage is of great importance for appropriate data collection and interpretation. Thromboxane $A_2$is generated *in vivo* from platelets upon activation. It is a short-lived prostanoid in biological fluids, since it undergoes a fast, spontaneous, non-enzymatic transformation into TXB_2, stable but biologically inactive, which is then enzymatically transformed in the human liver into two stable and inactive 11-dehydro-$TXB_2$and 2,3-dinor-TxB_2which are excreted un-modified in the urine. To measure 11-dehydro-TXB_2(TXM) in the urine, the lipid fraction is extracted by chromatography and measured in the extracts. In this study we tested the TXM in the extracts of urine samples, over 60 months. We also checked the stability of urine samples by measuring urinary creatinine in the same time interval.

Methods: One-ml urine samples were extracted by a validated chromatographic method (Pradelles et al., Anal Chem. 1985; Pagliaccia et al., Clin Lab. 2014). Extracts were first assayed for TXM within 7days from the extraction procedure and then stored at -40°C in a freezer equipped with temperature recorder. TXM was measured by previously described enzyme-immunoassay (Pradelles et al., Anal Chem. 1985). Creatinine was assessed directly in urine samples by a commercial kit (Creatinine Colorimetric Detection Kit; Enzo Life Sciences, Farmingdale, NY). Urine samples were stored at -40°C between the first and the second creatinine determination. Extracts or urine samples were assayed for a second time over a storage interval spanning from 1week up to 60 months. Values were analysed as % of the first measurement or as absolute values, as appropriate.

Results: Four-hundred and sixty urinary extracts were assayed twice for TXM, between one week and over 60 months after the extraction. The TXM absolute values were highly and linearly correlated between the first and the second measurement (rho: 0.95, $p<0.0001$). TXM values expressed as % of the first value did not show any trend of significant changes over time (rho:-0.002; $p=0.95$). Two-hundred forty-nine urine samples were assayed twice for creatinine between 1week and 60 months. For each sample, the two measurements were highly and linearly correlated (rho: 0.91, $p<0.0001$). Urinary creatinine values expressed as % of the first value did not show any trend of significant changes over time (rho: 0.01; $p=0.80$). TXM and creatinine data are reported in the Table.

Biomarker	0-6months % of first measurement	7-12months % of first measurement	13-24months % of first measurement	25-36months % of first measurement	36-60 months % of first measurement
TXM (n =460)	99.7±13.1% (n=283)	97.8±17.1% (n=45)	99.9±15.7% (n=48)	103.2±18.1% (n=47)	96.4±15.2% (n=37)
Creatinine (n=249)	97.3±15.9% (n=16)	99.53±16.1% (n=41)	100.3±16.3% (n=57)	96.2±17.1% (n=86)	97.9±19.9% (n=49)

Conclusion: TXM values in urinary extracts and creatinine values in urine samples appear to be stable up to 60 months when stored at -40°C. These data may be relevant when measuring biomarkers in samples long-term stored in biobanks and thawed over several years.