**Tackling the preanalytical instability of dihydropyrimidine dehydrogenase phenotyping via dried blood microsampling**

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**Background:** Dihydropyrimidine dehydrogenase (DPD), a crucial enzyme in pyrimidine metabolism, catalyses the rate-limiting step in the degradation pathway of the chemotherapeutic 5-fluorouracil (5FU). More importantly, DPD accounts for over 80% of 5FU catabolism, making enzyme deficiency a risk factor for severe toxicity, including myelosuppression. To avoid severe toxicity, screening for (partial) DPD deficiencies prior to fluoropyrimidine therapy is increasingly implemented into clinical practice. Both genotyping and phenotyping are currently performed, the latter being preferred due to a greater sensitivity. DPD phenotyping encompasses quantification of the endogenous substrate uracil in plasma, with increasing concentrations indicating enzymatic deficiency. A drawback of this approach lies in the preanalytical phase, as uracil concentrations rapidly increase after blood sampling. Thus, samples not handled according to the guidelines can give rise to false positive results.
**Methods:** As analyte stability is often improved in a dried matrix, this study -which is still ongoing- evaluates the role of conventional dried blood spots (DBS) in improving the preanalytical issues of uracil determination. An efficient extraction protocol for DBS was set up, along with optimization of a liquid chromatography – tandem mass spectrometry method. The preanalytical phase was assessed by a one-to-one comparison of uracil in both liquid (blood & plasma) and dried matrices (DBS). Briefly, 20µL blood and plasma samples were frozen at -80°C immediately after collection (=T0), while 20µL DBS were generated simultaneously. Remaining blood and plasma were left at room temperature (RT), and aliquots were stored at various time points up to 24h. DBS samples were dried for at least 3h (=T0) and stored similarly. Also, DBS subjected to 60°C for two days, mimicking thoroughly dried/aged samples, were analysed.
**Results and conclusion:** Uracil signals were compared to T0 for each matrix. As expected, blood and plasma showed increasing signals over time, reaching 159%±8 and 122%±5, respectively, after 24h at RT. In contrast, no increase was observed for DBS samples even after 24h storage at RT. Lastly, the results obtained for aged samples were also within 20% deviation compared to fresh DBS. This study suggests that dried blood microsampling is a promising solution for preanalytical challenges in DPD phenotyping.
**Keywords:** microsampling, uracil, DPD, phenotyping, stability