Impact of the preanalytical practices on the uracil and dihydrouracil stability for the diagnosis of dihydropyrimidine dehydrogenase deficiency. Maillard M.[1], Langevin M.[2], Ciccolini J.[3], Etienne MC.[4], Tafzi N.[5], Launay M.[6], Richard D.[7], Gautier E.[8], Royer B.[9], Narjoz C.[10], Haufroid V.[11], Bouges H.[4], Picard N.[5], Barin-Le Guellec C.[2,12], Thomas F.[1,13]

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Background: EMA's safety committee has recently recommended that patients should be tested for dihydropyrimidine dehydrogenase (DPD) deficiency before starting cancer treatment with 5-FU and related medicines (capecitabine and tegafur). Phenotypic activity of DPD relies on the determination of the uracil (U) and its metabolite, the dihydrouracil (UH2) in plasma. Compliance with pre-analytical conditions is essential for the reliability of the results because of the low stability of U and UH2. This study aimed to provide unpublished data of stability of U and UH2 by French and Belgian laboratories during assay development.

Methods: Plasma concentrations of U and UH2 were collected from ten French and one Belgian laboratories. Several preanalytical conditions were tested as time between whole-blood collection and centrifugation, stability in plasma stored during variable periods and temperatures, types of sampling tubes, long-term freezing, freeze/defrost cycles. Intraindividual variability of U and UH2 concentrations was also assessed in patients that have been analyzed at 2 different timepoints.

Results: data analysis of stability in whole-blood revealed that U and UH2 in samples stored at 4°C during at least 12 hours did not exceed ±20% of the reference relative concentration (n = 18 samples). On the contrary, when stored at room temperature (RT), U rapidly increased 1h30 after sampling (+35%), whereas UH2 was stable until 6 hours (+21%). At 12 hours, both U and UH2 were higher (+205% and 98%, respectively). At 4°C, UH2 in plasma was stable, while U started to increase at 18 hours (+33%). In plasma after defrosting, U and UH2 seemed stable for 48h at 4°C and RT. Long-term freezing did not impact U (-5%) and UH2 (+5,7%) after one year. Finally, intraindividual variability was evaluated in samples complying with the recommended time between collection and centrifugation. Mean coefficients of variation between the 2 values of U, UH2 and UH2/U of a same patient were of 24%, 18%, and 19%, respectively (n = 75 patients).

Conclusions: these unpublished experimental results together with a review of the literature will allow giving recommendations concerning samples handling for DPD phenotyping by uracilemia determination in plasma.

Key Words: 5-fluorouracil, dihydropyrimidine dehydrogenase, preanalytical conditions