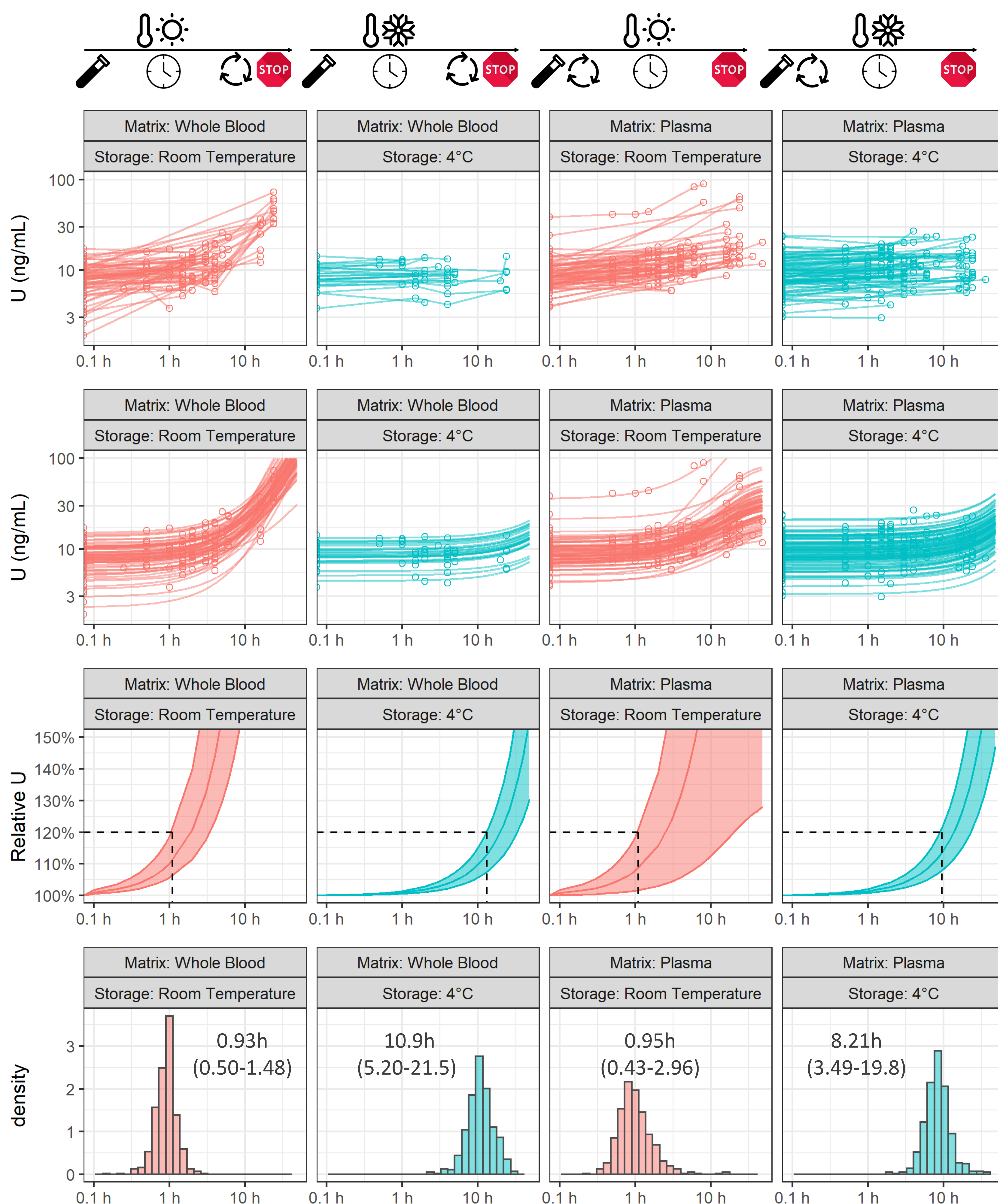


Non-linear mixed-effects modeling (NLME) to evaluate the pre-analytical stability of uracil in the context of dihydropyrimidine dehydrogenase deficiency testing

Context

- **Dihydropyrimidine dehydrogenase (DPD)** deficiency testing is mandatory in France and recommended by the European Medicines Agency before initiating a fluoropyrimidine (FP)-based treatment in cancer patients. This consists in measuring the **uracil plasma concentration (U)**, the physiological substrate of DPD. For example, a partial deficiency is defined as $U > 16$ ng/mL and justifies decreasing FP dose.
- Strict **pre-analytical conditions** must be respected to guarantee test interpretation since **U increases** if blood is not **immediately centrifuged** and stored at -20°C until being analyzed.
- To provide robust guidelines for this pre-analytical process, several laboratories have conducted their own **stability studies**, but they lack rigorous methodology: not respecting **identical times** across studies, using **small data size**.

Objective: Provide recommendations for the pre-analytical process of uracil measurement with NLME



Data collection

U was determined on residual aliquots of patients sampled for routine DPD deficiency testing in multiple centers. **Stability before or after centrifugation** (whole blood or plasma) was explored, either at a **cold (4°C)** or at **room temperature (RT)**, and at various times (minimum twice, from 30 min to 48 h post-sampling). 241 profiles (626 U values) from seven hospitals were available for analysis.

Modeling

Data of U vs length of time until -20°C storage was analyzed longitudinally with a **population approach** where each sample was considered as an individual (FOCE+I algorithm, NONMEM 7.4). Profiles at RT were fitted using a **Gompertz model** to capture the **saturation of U increase**. Profiles at 4°C were fitted using an exponential increase model. The difference between whole blood and plasma was implemented using different fixed effects parameters. Inter-individual variability was set as log-normal, and residual error as additive on log-transformed U. See Table 1.

Simulation

Four cohorts (combining RT/ 4°C and whole blood/plasma) of 500 U vs time profiles were **simulated** (mrgsolve 1.0.0). The time period when **U did not increase by more than 20% in 95%** of the profiles was measured.

With Uncertainty

The simulation procedure was repeated 500 times by **varying the population parameters** to account for their uncertainty and to derive 90% confidence intervals. The covariance matrix obtained in NONMEM was used to implement uncertainty as **multivariate Normal on fixed effects parameters** and as **inverse-Wishart on random effects parameters**. These were generated with the R package **simpar** (0.1.0). The code created to derive the covariance matrix and the degrees of freedom (df) from the NONMEM output is available as a proper R package: **uncrntny**, accessible at: <https://github.com/FelicienLL/uncrntny>

Modeling & Simulations says: Maximum 1h at room temperature either as plasma or whole blood.

Conclusion

- Pre-analytical steps should be carried out within a **maximum of one hour** at RT to adequately measure U and to accurately detect DPD deficiency
- **Storage at 4°C increases sample stability**, but precise recommendations for time periods cannot be made due to the large 90%CI, explained by the **data sparsity** at later measurements.
- Non-linear mixed-effects modeling made it possible to analyze data collected from various centers using different collection times to assess stability.
- It illustrates an **original use** of a population approach outside the domains of PK/PD and disease modeling.

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Table 1. Population parameters	Parameter	Estimate	Precision	Uncertainty
Fixed effects			(CV %)	(variance)
Initial U	U0 (ng/ml)	8.68	2.6%	0.0486
Growth constant in whole blood	K_WB (h^{-1})	0.0317	7.4%	5.49e-06
Growth constant in plasma	K_PLS (h^{-1})	0.0615	41.6%	5.89e-04
Effect of 4°C storage on K_WB	COLD_K_WB	0.293	29.7%	7.58e-03
Effect of 4°C storage on K_PLS	COLD_K_PLS	0.197	42.5%	7.96e-03
Carrying capacity in whole blood	UMAX_WB (ng/ml)	200	FIXED	10000
Effect of plasma on UMAX	MATRIX_UMAX	0.160	32.2%	2.34e-03
Random effects				(df)
Inter-individual variability in U ₀	ω U0 (CV%)	36.9%	6.6%	116
Inter-individual variability in K	ω K (CV%)	33.4%	34.2%	5
Inter-individual variability in U _{MAX}	ω UMAX (CV%)	64.9%	19.1%	14
Residual error	σ U (CV%)	13.7%	6.1%	N/A