Comparison between the analytical requirements of ThDP and ETKA[2]

	ThDP	ETKA
Sample Type	- Requires venous blood collected into EDTA- or heparin-containing specimen tubes, and can be measured in erythrocytes or in whole blood - Whole blood ThDP requires less sample processing - For erythrocyte ThDP analysis, the cells must be washed with isotonic saline solution (see the details on the right)	- Requires venous blood collection; washed, anticoagulated (heparin or EDTA) erythrocytes are used for this assay - Erythrocytes are washed three times with isotonic saline solution (0.9% NaCl) to avoid osmotic damage to the cells
Sample Storage	Thiamine is stable for a few hours refrigerated; for a few months stored frozen at -20°C; and several months stored frozen at -80°C.[67]	Washed erythrocytes, without supernatant, should be stored in at -80°C, but can be stored in a refrigerator for a few hours prior to freezing.
Analytical Techniques	Can be measured directly using one of two liquid chromatography techniques: - High performance liquid chromatography (HPLC) with either pre- or post-column derivatization coupled with fluorescence detection[68]: samples are prepared by removal of proteins and derivatization to produce fluorescent thiochrome compounds that are separated on a reverse phase analytical column, then detected and quantified.[67] - Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)[69]: allows sensitive and selective measurement of underivatized thiamine diphosphate.	Measured with a UV-vis spectrophotometer at an absorbance of 340 nm in a UV-transparent 96-well microplate. All wells must be held at the same temperature, 37°C, and all reagents must be in excess throughout the temperature equilibration and reading phases to ensure linearity.
Minimum Volume	- For HPLC: 300-500µL of erythrocytes or whole blood - For LC-MS/MS: 150-250µL of erythrocytes or whole blood	Minimum volume is 30 µL of washed erythrocytes. To aid sample handling and to allow repeat analysis a minimum of 200µL is recommended. In practice the process is easier with larger samples, > 1mL of whole blood is suggested.

	ThDP	ETKA
Quality Control and Standards	External standard: a 1mM stock solution of ThDP should be prepared in 0.1M HCl and stored at -80°C. Working standard solutions should be prepared fresh by dilution the stock solution to concentrations of 0, 25, 50, 100, 200, 400, 600nmol/L. Internal standards: to account for losses during sample preparation, while desirable, these are rarely used due to the lack of appropriate thiamine standards useful for HPLC/fluorescence methods. Isotopically-labeled internal standards are available for LC-MS/MS methods. Calibrators and QC material (as lyophilised whole blood) are available (e.g. from Chromsystems (Germany) and Recipe (Germany)). The Royal College of Pathologists of Australasia (RCPA) runs a quality assurance program (QAP) for thiamine.	The assay does not require a calibrant. Quality control specimens should be prepared from bulk samples from single donors and stored at -70°C; the between-assay coefficient of variation (SD*100/mean) of controls in the "adequate status" range is typically 3 to 5%.
Data Analysis and Presentation	- Erythrocyte ThDP concentrations should be reported in nmol/L red blood cells (RBCs). - In whole blood ThDP analysis, there is a need to normalize to RBC volume or hemoglobin concentrations.[70] Best practice would be to present both the measured ThDP (nmol/L whole blood) and the ThDP normalized to hematocrit (nmol/L RBCs) or hemoglobin (nmol per gram hemoglobin). This will ensure that data from different studies are comparable and allow hematocrit or hemoglobin normalized whole blood data to be directly compared with erythrocyte ThDP concentrations.	- ETK activity is expressed in terms of the rate of decrease of absorbance at 340 nm, corrected for any changes in the reagent blank. - The ratio of the absorbance in the presence and absence of exogenous ThDP gives the ETK Activation Coefficient (ETKAC), i.e. ETK activity with added ThDP / baseline ETK activity. - ETKAC should be reported and is sometimes expressed as the percentage activation α. - Alternatively, basal ETK activity per unit mass of hemoglobin (micromoles/min/gHb) may be reported.

	ThDP	ETKA
Suggested* Cut-offs *Note: different cut-offs have been proposed and there is no agreement on the most adequate values.*	Whole blood: - 70-180 nmol/L: healthy range - <70 nmol/L: deficiency Erythrocytes[71]: - 120-150 nmol/L: mild deficiency - <120 nmol/L: deficiency	ETKAC values and risk of clinical thiamine deficiency: - ≤1.15 (α≤15%): low risk - 1.15–1.25 (α15%-25%): moderate risk - >1.25 (% activation α>25%): high risk Alternatively, the proposed cut-offs for thiamine deficiency using basal ETKA (in micromoles/min/gHb) are[15]: - Infants: ≤0.59 - Females aged 4–18y: ≤0.57 - Females aged 19–24y: ≤0.57 - Females aged 25–34y: ≤0.50 - Females aged 35–49y: ≤0.47
Challenges and Limitations	Pre-analytical: - Specimens should be protected from light and frozen at -80°C. - Specimens of whole blood must be frozen to ensure lysis of erythrocytes. Analytical: - Chromatographic separation of thiamine metabolites is necessary for analysis by HPLC with optical detection. This is not necessary for determination by mass spectrometry. - HPLC requires derivatization of the thiamine species, which is not required for LC MS/MS. - Analytical methods have not been standardized. Considerable variation has been observed between laboratories.[72]	Pre-analytical: - As freezing causes erythrocyte lysis, the erythrocyte washing must be completed prior to freezing. - Fresh-frozen specimens must be used, freeze thaw cycles can diminish the transketolase activity. Multiple aliquots should be prepared and stored in the event that a sample needs to be re-analyzed. Analytical: - Maintaining uniform temperature across the plate is required for each enzyme assay procedure - The assay can be difficult to standardize, and inter-assay precision can be poor without careful analytical procedures. Interpretational: ETKA can be influenced by factors other than ThDP concentration, such as age, genetics, and variability in binding of the apoenzyme.[73]

Thiamine diphosphate (ThDP); erythrocyte transketolase activation coefficient (ETKAC); ethylenediaminetetraacetic acid (EDTA); high performance liquid chromatography (HPLC); liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS)