

## Assessment of thiamine status in humans

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### Abstract

Thiamine (vitamin B<sub>1</sub>) is an essential nutrient with established adverse health consequences associated with acute deficiency. Emerging evidence also suggests long-term neurocognitive deficits due to sub-clinical deficiency in early life, and there is a growing appreciation of a potential role for thiamine in Alzheimer's disease. Therefore, the assessment of thiamine biomarkers, particularly in large, population-based studies, is critical to grasp the true scope of this public health problem.

The most reliable biomarkers of thiamine status are whole blood concentrations of thiamine diphosphate (ThDP) measured by high performance liquid chromatography (HPLC) with fluorescence detection or liquid chromatography tandem mass spectrometry (LC-MS/MS), and the functional assay of transketolase activity (erythrocyte transketolase activity coefficient, ETKAC). Plasma thiamine reflects recent intake, and is not a useful biomarker of thiamine status.

This manuscript describes the different biomarkers and methods of assessment, their advantages and disadvantages, and related cutoffs for deficiency. A table of 'performance characteristics' is provided to guide users on the quality and performance characteristics of each assay, and practicalities related to their cost, resource requirement, and maintenance. Provision of these insights will help inform decisions about inclusion of thiamine analysis in future work to better understand the extent of global thiamine deficiency.

## 1. Thiamine (vitamin B<sub>1</sub>)

### 1.1 Introductory remarks

Thiamine (vitamin B<sub>1</sub>) is an essential water-soluble vitamin that has re-gained attention in recent years as a neglected nutrient of public health concern in low- and middle-income countries (LMIC) (Brown et al., 2025; Johnson et al., 2019). The diphosphorylated form of thiamine (thiamine diphosphate, ThDP) is a coenzyme in energy metabolism pathways for carbohydrates and branched-chain amino acids, as well as for neurotransmitter synthesis, so thiamine deficiency can cause reduced ATP synthesis, oxidative damage, and impaired neurological function (Whitfield et al., 2018). More recently, suboptimal thiamine intakes during infancy have been implicated in long-lasting neurocognitive delays, particularly in gross motor and language

domains (Fattal-Valevski et al., 2005; Mimouni-Bloch et al., 2014). Given these vital roles in human health and development, accurate assessment of thiamine status is essential to spur clinical treatment among individuals, and to design public health interventions and policies at a population level.

Thiamine is required in relatively small quantities, with recommended nutrient intakes of 1.1 mg/day and 1.2 mg/day for women and men, respectively (WHO & FAO, 2004). However, with limited body stores and a short half-life, routine intake of thiamine is necessary to prevent deficiency (Whitfield et al., 2018). Inadequate thiamine intakes are common among groups consuming limited, carbohydrate-rich diets, as much of the thiamine in cereals is removed in processing, and thiamine requirements increase with increasing carbohydrate intakes. Meat, particularly pork and organ meats, whole grains, and legumes are high in thiamine, while thiamine antagonists include betel nuts and tea, and thiaminase-containing raw fish, ferns, and African silkworm larvae (Whitfield et al., 2018). Excess thiamine intakes are not thought to be of concern because of rapid renal clearance of excess thiamine (WHO & FAO, 2004).

### ***1.2 Importance of assessing thiamine status***

A recent modeling analysis estimated that 30% of the global population – or 2.2 billion people – have inadequate thiamine intakes (Passarelli et al., 2024). Thiamine deficiency has most commonly been associated with Southeast Asia, as beriberi was discovered in the Dutch East Indies (Indonesia) (Carpenter, 2000). While thiamine remains a nutrient of concern in the region, with recent reports of thiamine deficiency in Cambodia, Myanmar, and Laos PDR (Smith & Hess, 2021), there is growing evidence of thiamine deficiency beyond Southeast Asia, including reports from India (Hilal Ahmad et al., 2019; Koshy et al., 2018; Koshy et al., 2021), the Pacific Island nation of Kiribati (Green et al., 2021; Nilles et al., 2018), and throughout Africa (Johnson et al., 2019; Bourassa et al., 2022). Given the widespread concern about thiamine intakes throughout LMIC around the globe, there is a need for biological data to corroborate deficiency. At present, assessment of thiamine status is not often included in population-representative nutrition biomarker surveys, so there is a limited understanding of the global burden of thiamine deficiency (Brown et al., 2025). Exceptions are the United Kingdom National Diet and Nutrition Survey (NDNS) (Koulman et al., 2026), and recent surveys in Madagascar and Nigeria (Rohner et al., 2026; Federal Government of Nigeria, 2024).

### ***1.3 At-risk populations for thiamine deficiency***

Thiamine deficiency manifests with several distinct clinical profiles (Smith et al., 2021). Historically, deficiency has been categorized by the type of beriberi: wet beriberi (cardiac presentation), dry beriberi (neurological symptoms), shoshin beriberi (rapid, acute clinical presentation), and infantile beriberi, or as Wernicke-Korsakoff syndrome with presentation among individuals with alcohol use disorder. Given the broad and overlapping symptoms of deficiency, ‘thiamine deficiency disorders’ (TDD) was coined to capture the broad spectrum of adverse outcomes (Whitfield et al., 2018).

Thiamine deficiency is generally uncommon in high-income settings, except among certain clinical populations (Rosewarne & Farina, 2026; Marrs & Lonsdale, 2021). Individuals with

alcohol use disorders are the most common risk group for thiamine deficiency, which presents as Wernicke-Korsakoff syndrome (Frank, 2015). Excessive alcohol consumption can impair thiamine absorption at a time of heightened thiamine requirements and often limited dietary thiamine intake (Gomes et al., 2021). Post-operative bariatric surgery patients are at risk due to limited nutrient intakes and/or vitamin malabsorption in cases of bypass or removal of the proximal small intestine (Frank, 2015). Finally, thiamine is vital in refeeding, including in cases of disordered eating as well as in the treatment of severe acute malnutrition (Hiffler et al., 2017; O'Brien et al., 2025). As such, the ability to measure thiamine status is important in clinical settings.

In LMIC settings, a monotonous diet reliant on a refined, non-fortified carbohydrate staple food is the greatest predictor of low thiamine status (Whitfield et al., 2018). Thiamine requirements increase with higher physical activity, and during periods of high metabolism, so groups such as pregnant/lactating women and labourers are at higher risk of deficiency. For instance, an outbreak of beriberi was reported among otherwise healthy young men working as illegal gold miners in French Guiana (Mosnier et al., 2017); here and in similar outbreaks, thiamine deficiency was explained by overall low dietary intake, a reliance on washed, polished rice, and heavy physical exertion (Doung-ngern et al., 2007; de Montmollin et al., 2002).

While thiamine deficiency is concerning at any lifestage, a major focus of recent research has been on infants (Gallant et al., 2021; Smith et al., 2024), given their comparatively high requirements relative to small body size and low food intakes (Whitfield et al., 2018; Fattal-Valevski 2011). Infantile thiamine deficiency normally presents in the first six months, which is the exclusive breastfeeding period when the only source of thiamine is mother's milk (Luxemburger et al., 2003; Smith et al., 2024). The symptoms are fairly generic, including tachypnea, tachycardia, dysphonia, edema, cyanosis, colic, and vomiting (Smith et al., 2024), so infants may die before medical treatment is ever sought. Lactating mothers should be targeted to prevent infantile beriberi, as women with low thiamine intakes produce milk low in thiamine (Dror & Allen 2018; Whitfield et al., 2018).

Infantile beriberi can be fatal, but even deficiency not severe enough to cause clinical presentation may have severe and long-lasting consequences. An unfortunate 'natural experiment' among Israeli infants resulted from a manufacturing error that erroneously excluded thiamine from infant formula (Fattal-Valevski et al., 2005). These otherwise well-nourished infants had low thiamine intakes in early life. Although many of these infants did not develop clinical beriberi symptoms, follow-ups through childhood highlighted persistent language and motor delays compared to their peers (Fattal et al., 2011; Harel et al., 2017; Mimouni-Bloch et al., 2014). This recent evidence suggests that sub-clinical thiamine deficiency may put millions of infants at risk of neurocognitive deficits, undercutting life-long well-being. Similarly, there is growing interest in the role of thiamine in dementia and Alzheimer's disease (Gibson et al., 1988; 2016).

Given the serious adverse outcomes of thiamine deficiency, and large geographic footprint of LMIC at risk of low dietary thiamine intakes, the assessment of thiamine biomarkers, particularly in large, population-based studies, is critical to grasp the true scope of this public health problem. Below we will highlight the most common thiamine assessment methods for

blood, providing insights to help inform decisions about inclusion of thiamine analysis in future work.

## **2. Methods for thiamine assessment in human blood samples**

### ***2.1 Introductory remarks***

Detection of thiamine and its vitamers in body fluids requires very sensitive methods. For instance, ThDP concentration in whole human blood is approximately 100 nM. Thiamine and thiamine monophosphate (ThMP) concentrations are approximately one order of magnitude lower. Typically, thiamine vitamers in biological samples can be separated by high performance liquid chromatography (HPLC) and detected either as fluorescent thiochrome derivatives or by mass spectrometry. Indeed, during the last two decades, powerful liquid chromatography tandem mass spectrometric (LC-MS/MS) methods were developed, allowing the detection of all thiamine derivatives and provitamins (Kim et al., 2019; Sambon et al., 2020). These methods, while more sophisticated than fluorescence-based HPLC methods, also require skilled technicians and the instrumentation is highly expensive.

A third common method exists, a functional assay for thiamine status assessment, the erythrocyte transketolase activity coefficient (ETKAC). Unlike HPLC and LC-MS/MS methods that report a direct assessment of thiamine concentrations in blood, this functional biomarker assesses the degree of saturation of the ThDP-dependent enzyme transketolase, providing insights into thiamine status of the individual (Jones et al., 2021).

Many variations of the general methods described here have been developed for a rapid, cost-effective, and routine determination of specific vitamers for the estimation of the thiamine status in humans (Vachali et al., 2025). Hence the choice of the method depends on factors such as the derivative to focus on, cost, and laboratory environment. Each of the methods is summarized below, alongside **Table 1**, where these methods are compared using numerous performance and logistical considerations.

### ***2.2 Specimen Requirements for HPLC and LC-MS/MS***

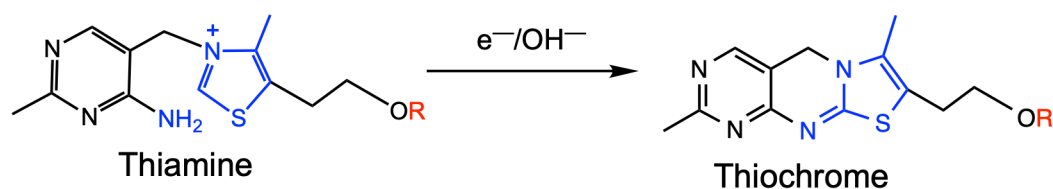
Whole blood specimens collected using heparin or ethylenediaminetetraacetic acid (EDTA) as anticoagulants are suitable for direct measurement using chromatographic methods (HPLC, LC-MS/MS). Thiamine is photosensitive and specimens must be protected from light during storage and transport to the laboratory. Samples should be frozen at -70°C to ensure complete lysis of erythrocytes. Samples frozen at -20°C may yield falsely low thiamine concentrations. Plasma thiamine concentrations reflect recent intakes rather than thiamine nutritional status.

### ***2.3 HPLC***

Measurement of thiamine and its derivatives by HPLC involves separation by liquid chromatography typically achieved with a C18 chromatography column (Basiri et al., 2016; Lu & Frank, 2008; Zhang et al., 2017), followed by fluorescence detection.

### 2.3.1 Derivatization

UV detection of thiamine is neither sensitive nor selective enough, and is only useful in relatively pure pharmacological preparations. The most sensitive HPLC methods are based on derivatization of thiamine vitamers in fluorescent thiochrome derivatives (**Figure 1**) followed by HPLC separation and fluorescence detection.



**Figure 1.** Oxidation of thiamine to the fluorescent thiochrome in alkaline medium. The amino function of the pyrimidine ring and the thiazolium cycle, essential for cyclization, are drawn in blue.  $R = H$ ,  $PO_3^{2-}$  (ThMP) or  $P_2O_6^{3-}$  (ThDP).

Various HPLC techniques have been described (Basiri et al., 2016; Bettendorff et al., 1991; Lu & Frank, 2008).

Typically, thiamine derivatives are oxidized by potassium ferricyanide in a strongly alkaline medium, hence the importance of neutralization to  $pH > 5$  of the sample extract. Moreover, the formation of a thiochrome requires an intact thiazolium ring. Precolumn and post-column derivatization procedures have been described. According to our experience precolumn derivatization is easier, but in that case thiochrome derivatives are separated on the analytical column. As they are fluorescent only under alkaline conditions, an alkali-resistant column must be chosen.

For post-column derivatization, thiamine derivatives are separated on the column, but a further pump is required for injecting the oxidant into a mixing chamber with the column efflux. We found that postcolumn derivatization was more cumbersome and more subject to system failure than precolumn derivatization, mainly because of the high content of sodium hydroxide in the oxidant solution. A spectrofluorometer is to be preferred over a filter fluorometer because of a much better selectivity. Hence, some fluorescence-based HPLC methods are able to simultaneously determine thiamine, ThMP, ThDP, thiamine triphosphate, and adenosine triphosphate in human tissues (Gangolf et al., 2010).

As thiamine derivatives are present in sample solution at very low, submicromolar, concentrations, suitable standard solutions must be prepared in the same concentration range and particular attention must be paid for the material used for preparing the sample and further processing in order to avoid absorption of the material. According to our experience, polypropylene was neutral in that sense, but glass should be avoided.

Furthermore, recent data suggest that a non-negligible portion of ThDP can exist in an oxidized form in animal tissues (Pavlova et al., 2021), as well as in humans exposed to high doses of radiation. Indeed, one study found that ThDP was predominantly in an oxidized form in the

blood of Chernobyl accident liquidators (Donchenko et al., 1997). Such forms, likely open-ring thiazole disulfide derivatives, require specific detection methods. Presently, both the biological role of these derivatives and their potential significance in assessing human thiamine status remain unknown.

### *2.3.2 Extraction*

Many procedures are described in the literature for the extraction of thiamine and its vitamers from biological samples. As thiamine as well as all its natural vitamers are water-soluble, extraction can easily be realized by homogenization of the tissues in trichloroacetic acid (TCA) at a final concentration of 10 - 12% (with less, thiamine extraction may only be partial and with more acid, its elimination becomes challenging). TCA can easily be extracted with 3 times 3 volumes of water-saturated diethylether (preferably HPLC grade without stabilizer, which may interfere with fluorometric determination).

## **2.4 LC-MS/MS**

From **Figure 1** it is evident that only thiamine derivatives with an intact thiazolium ring can be transformed into a thiochrome derivative. Therefore, several methods based on mass spectrometry detection have been developed (Kim et al., 2019; Verstraete et al., 2020). These techniques have the advantage of allowing the detection of derivatives with an open thiazolium ring that cannot be transformed into a fluorescent thiochrome (Sambon et al., 2019, 2020). However, mass spectrometric detection can also be used for the detection of conventional thiamine derivatives (i.e. thiamine, ThDP). Mass spectrometry methods are sensitive and selective. The complex sample preparation processes required for HPLC assays are not necessary, which shortens analysis time, and isotope-labelled compounds are available as internal standards to compensate for variability introduced during extraction and analysis.

### *2.4.2 Extraction*

Frozen specimens are thawed at ambient temperature for two hours to ensure optimum recovery of ThDP. LC-MS/MS allows use of a stable-labelled internal standard, such as ThDP-d<sub>3</sub>. Sample preparation can be as simple as protein precipitation with TCA followed by mixing and centrifugation prior to analysis. (Vachali et al., 2025)

## **2.5 ETKAC**

The cytosolic transketolase catalyses two essential reactions of the non-oxidative (reversible) branch of the pentose phosphate pathway. It is essential for recycling ribulose 5-phosphate (precursor of ribose 5-phosphate) towards the glycolytic pathway (Li et al., 2019). Being central to metabolism, transketolase is present in all cells, including erythrocytes, providing an accessible tissue for the study of thiamine status.

Activity of the transketolase enzyme in erythrocytes has been used to assess thiamine status since the 1960s (Brin, 1962). Erythrocytes cannot synthesise proteins thus the transketolase levels represent protein production during precursor cell synthesis (Edwards, 2026). In the assay,

transketolase activity is therefore largely determined by the availability of ThDP. The essence of the assay is simultaneous or consecutive measurements of transketolase activity in the absence of exogenous ThDP (basal activity rate) and with the addition of exogenous ThDP (activated rate). From the calculation of the 'activity coefficient' or the ratio of these two measurements, the erythrocyte transketolase activity coefficient (ETKAC) is derived. An ETKAC value near 1 indicates almost complete saturation of the transketolase enzyme and suggests functional thiamine sufficiency. A higher ratio indicates the degree of activation of the transketolase enzyme through the addition of exogenous ThDP. The greater the ratio, the greater the indicated deficit in ThDP. The assay is typically performed in erythrocytes washed in saline to remove white blood cells.

Early forms of the assay used colorimetric changes to determine transketolase activity whereas current assays monitor the rate of NADH oxidation by spectrophotometry (Edwards, 2026; Jones et al., 2021). Methods have evolved over the decades from test tube based, to automated clinical chemistry analyzers, with more recent methods utilising a 96-well microplate format for convenience and high throughput (Jones et al., 2021). In the assay, transketolase catalyses the metabolism of two reactions in the pentose phosphate pathway. The resultant glyceraldehyde 3-phosphate is metabolized to dihydroxyacetone phosphate, which in the final reaction is reduced to glycerol-3-phosphate by glycerol-3-phosphate dehydrogenase and NADH (Edwards, 2026; Jones et al., 2021).

A protocol published with the aim to standardise the transketolase activity assay has been used in a several studies, randomized controlled trials (Bourassa et al., 2022; Gallant et al., 2021; Hess et al., 2024; Koulman et al., 2026) and nutritional surveys of micronutrient status (Rohner et al., 2026; Federal Government of Nigeria, 2024). Measurements are performed with a standard microplate reader available in many laboratories. This protocol has also been adopted and applied in other laboratories (Bandyopadhyay et al., 2025; Hauber et al., 2026; Therrien et al., 2026). The transketolase assay has also been applied to other tissues and species (Gibson et al., 1988; Giguère & Butterworth, 1987; Hauber et al., 2026; Therrien et al., 2026; Zhao et al., 2009) however, as recently discussed, consideration must be given to the assay conditions specific to each application beyond erythrocytes (Edwards, 2026).

The primary outcome of the assay is the ratio of activated to basal transketolase activity (ETKAC). Commonly, assay results are expressed as a percentage, typically known as the TPP effect. Results may also be expressed as the latency (the inactivated fraction of transketolase) (Edwards, 2026; Jones et al., 2021).

The activity coefficient, TPP effect, and latency all describe the relative change in enzyme activity with and without exogenous ThDP. Whilst this is an advantage in terms of the robustness of the assay and minimises the impact of differences in the amount of enzyme it may become a limitation where differences in enzyme and/or tissue are systematically different between test groups. Where the apoenzyme is lower, as has been hypothesised to occur in long-term thiamine deficiency, less ThDP is required to stimulate the transketolase and therefore a ratio and TPP effect in the normal range may be observed. In this scenario, measurement of the absolute basal activity can provide additional information. However, further measurements and calculations are required to determine the absolute basal activity in units of enzyme activity, as well as

measurements of either hemoglobin or protein in the tested sample. A protocol for the calculation of basal activity has been published (Jones et al., 2023), and a protocol for expressing brain basal transketolase activity normalized to total protein is included in this volume (Parkington et al., 2026).

## ***2.6 Decision making tips for method selection***

The primary aim of this manuscript is to compare the different assays to assess thiamine status and to guide users on the most appropriate assay for a given setting, whether primarily research or clinical. The elements in **Table 1** provide guidance on the consumables and equipment for specific assays, their relative costs, the expertise associated with each, and other considerations such as the types of waste produced by each approach. Availability of equipment and staff, and accessibility of additional specialist support will clearly play a considerable role in determining the most feasible approach.

Three methods have been described, HPLC, LC-MS/MS and transketolase activity that align with current available and recommended assays (Brown et al., 2021). As alluded to above, there are primarily two types of micronutrient biomarker that provide information on nutritional status; those based on concentration and those related to metabolic function. This distinction is important and it is not the case that the two approaches necessarily give complete agreement. ThDP concentration measured by HPLC or LC-MS/MS as described indicates the level of this form of thiamine circulating in the blood, whether metabolically available and active, or not. In contrast, the transketolase assays (both ETKAC and the basal transketolase activity) provide information on the functional capacity of the ThDP-dependent enzyme at the tissue level. Each status biomarker has been demonstrated to be responsive to thiamine depletion and supplementation (Brin, 1964; Gallant et al., 2021; Haro et al., 1966; Wood et al., 1980; Baines & Davies, 1988). Studies assessing agreement between them have reported mixed results, partly due to the challenges of analytical variability in both biomarkers and because measurements conducted in groups with little or no thiamine deficiency (Baines & Davies, 1988; Bailey et al. 1994). Whilst some studies observed no relationship between biomarkers (Bailey et al 1994), others have found associations between ThDP and basal transketolase activity and/or ETKAC (Baines & Davies, 1988; Fidanza et al., 1989; Talwar et al., 2000). In terms of classification of thiamine deficiency, ThDP and basal transketolase activity performed better than between ThDP and ETKAC (Baines & Davies, 1988).

There are no point-of-care or field-friendly methods to assess thiamine status, so in common to each of the three approaches are the requirement for laboratory facilities with reliable electricity and water supplies, standard computing resources, and support for waste management streams to manage biological and chemical waste. Several groups have explored the use of dried blood spots (and microsampling devices) as an alternate sample collection method to venous blood draws to address some of the challenges such as cold chain management, which can be particularly challenging in LMIC (Huang et al., 2020; Mathew et al., 2019; Verstraete & Stove 2021a, 2021b). However, dried blood spot methods still ultimately rely on HPLC or LC-MS/MS for analysis, so the considerations in **Table 1** still apply.

The major difference between the methods are the capital outlay and the cost of servicing and maintenance, in particular for LC-MS/MS which would preclude this approach for many laboratories. In addition, liquid chromatography for either LC-MS/MS or HPLC requires reliable supplies of solvents. However, LC-MS/MS and to a lesser extent HPLC in general provide extremely versatile bioanalytical platforms. HPLC and LC-MS/MS platforms are relatively common in clinical settings in higher-income countries and therefore setting up of chromatography-based ThDP assays may be a more viable option in such settings. In contrast, the measurement of transketolase activity requires relatively little specialist equipment or servicing. However, this approach does require specialist chemicals, albeit in relatively small quantities.

As discussed above, ThDP is primarily found in erythrocytes rather than plasma, thus either whole blood or erythrocytes are required for analysis. Whilst for LC-MS/MS and HPLC, whole blood can be frozen for subsequent analysis, for the transketolase assays, washed erythrocytes are the preferred matrix to ensure the absence of white blood cells which, being very metabolically active, can contain high levels of transketolase enzyme (Buttery & Pannall, 1985; Smeets et al., 1971). This requirement for washed cells can be challenging in situations where blood samples are collected in the field, nevertheless it is possible as demonstrated by a number of recent studies in LMIC (Bourassa et al., 2022; Gallant et al., 2021; Hess et al., 2024; Rohner et al., 2026; Federal Government of Nigeria, 2024). No reference method is available for the determination of ThDP in blood. Consequently, there are no accuracy-based external quality assessment (EQA) schemes. However, EQA schemes from the Royal College of Australian Pathologists Quality Assurance Programs (RCPAQAP) and INSTAND (Institut für Standardisierung und Dokumentation im medizinischen Laboratorium) are available, providing targets based on laboratory means for comparison of ThDP in whole blood. However, few laboratories measure or subscribe to these schemes and results can be dominated by commercially available assays. Such schemes are useful to understand between-laboratory variability and its causes (Hoad et al. 2013). Unfortunately, the situation is no better for transketolase assays where no EQA scheme exists. As more laboratories renew interest in thiamine assessment using this approach and published methods allow harmonisation, opportunities may arise for assessment of interlaboratory comparisons and future standardisation. Lack of harmonisation between methods and laboratories may also contribute to the difficulty and uncertainty surrounding cutoffs for thiamine deficiency as discussed in *Section 3*.

As discussed at the start of this section, in most scenarios local expertise and resources will determine the feasibility of methodologies with a laboratory. More broadly, studies may rely upon international collaborators to measure thiamine status and then the decision of which assays to use may become more complex. In terms of cost, for the reasons described above, transketolase assays are likely to be less expensive than those using LC-MS/MS, and HPLC an intermediate option. Ideally, to build an evidence base, studies may choose to use both concentration and functional biomarkers. Previously published or locally available data may already be available for comparative purposes and may provide an incentive to use a particular method. ThDP concentration is a static measurement of thiamine status. On the other hand, and as has already been described, measurement of the transketolase activity provides a functional biomarker of thiamine status incorporating not only the availability of ThDP but also other factors such as enzyme availability and other cofactors (Edwards, 2026), some of which can be

captured by the more detailed assessment of basal transketolase activity. The converse of this holistic functional biomarker is that many other factors, in addition to thiamine, may also impact the results, ranging from physiological, genetic and nutritional factors to methodological and data-related factors (Edwards, 2026), although these are somewhat negated by the presentation of relative (ETKAC) rather than absolute results.

This manuscript has focused on blood thiamine status as a marker of population status and in relation to clinical deficiency. However, thiamine is also measured in other tissues, particularly the brain. Both chromatographic methods and the transketolase assay can be applied to tissues (Gangolf et al., 2010; Gibson et al., 1988; Klooster et al., 2013; Parkington et al., 2026). Measurements by HPLC showed that the distribution and concentration of thiamine forms vary significantly between human tissues (Gangolf et al., 2010).

### **3. Interpretative criteria**

Whilst it is well known that thiamine deficiency leads to the conditions described in *Section 1* above, there are other metabolic, physiologic and nutritional factors that have largely prevented the determination of cutoff values that can be used diagnostically. Of the methods shared here, ETKAC has the most agreed-upon cutoffs, with consortium groups commonly using ETKAC > 1.25 to define risk of thiamine deficiency, 1.15-1.25 to define marginal status, and ETKAC < 1.15 to define thiamine sufficiency (Institute of Medicine, 1998; Turck et al., 2016). But still, the evidence-base for these cutoffs is relatively small and largely derives from a handful of experimental studies and nutritional surveys conducted in the 1960s (Sauberlich, 1967).

There is no consensus around cutoffs for ThDP in blood. ThDP measurements by HPLC and LC-MS/MS can be made in erythrocytes (eThDP) or whole blood, the latter negating the requirement for blood processing, but the results may require correction for hemoglobin or hematocrit (Evliyaoglu et al., 2019; Caballero et al., 2025; Talwar et al., 2000). Using nationally-representative data from Cambodian Micronutrient Survey (linked with Cambodian Demographic and Health Survey 2014) where eThDP was measured on HPLC, Whitfield et al. reported status using numerous cutoffs from the literature (Whitfield et al., 2017). Depending on the cutoff employed, the prevalence of thiamine deficiency / low thiamine status among women of reproductive age (n=719) ranged from 27% (eThDP < 120 nmol/L) to 78% (eThDP <180 nmol/L) (Whitfield et al., 2017). The implications of the lack of cutoffs is obvious, as the urgency of action and subsequent policy solutions would differ whether one quarter versus three quarters of a population group were deficient.

Ideally, thiamine concentration cutoffs should be clinically meaningful, aligning with actual risk of clinical symptoms. A recent prospective cohort study in Lao PDR attempted to do exactly this: Hess et al. developed a clinical case definition for thiamine-responsive disorders among infants presenting to hospital, with blood samples taken before and after children were treated with supplemental thiamine (Hess et al., 2024). Those with favourable improvements in thiamine deficiency-related clinical symptoms were deemed thiamine-responsive, and hence would have been thiamine deficient upon hospital admission. Unfortunately, authors reported considerable overlap in ThDP concentrations between thiamine-responsive and non-responsive infants, so low sensitivity and specificity hindered their ability to set clinically predictive and meaningful cutoffs

(Hess et al., 2024).

In the absence of cutoffs, reference ranges are often used to estimate risk of deficiency (Marrs & Lonsdale, 2021). However, as Wilkinson et al. wrote of their use of reference ranges, “the lower limit of normal, seen in a healthy population, cannot be assumed to be the upper limit of abnormal” (Wilkinson et al., 1997).

#### **4. Summary and conclusions**

The measurement of thiamine biomarkers is critical for assessing individual and population thiamine status, and for understanding links between thiamine and health and disease. In this manuscript we describe currently available biomarkers and summarise their advantages and disadvantages. Despite the well-established dietary recommendations for thiamine, the risk of deficiency persists in many populations across the world. Emerging evidence of long-term neurocognitive deficits due to sub-clinical deficiency and a growing appreciation of a potential role for thiamine in the prevention and treatment of Alzheimer’s disease add to the need for reliable and well-characterised thiamine biomarkers.

The most reliable biomarkers of thiamine status are whole blood concentrations of ThDP measured by HPLC or LC-MS/MS, and the functional assay of transketolase activity. Plasma thiamine concentration is not a useful biomarker of thiamine status. The transketolase assay benefits from less expensive equipment and maintenance than other methods, and established cut-offs for deficiency and insufficiency. Efforts have been made to standardize the assay between laboratories, but cross-laboratory comparisons and materials for external quality control are lacking. HPLC is more expensive, a larger sample volume is required, and no suitable internal standards exist. LC-MS/MS provides advantages in sensitivity and specificity and isotopically-labeled internal standards are available, but equipment is expensive to purchase and maintain. External proficiency testing schemes are available and their use is encouraged to help understand differences between assays and laboratories. This is essential if progress is to be made on harmonising cutoffs for whole blood ThDP concentration, which is currently a major barrier to use of ThDP as a meaningful biomarker for health outcomes. However, in practice, the availability of equipment and technical staff are likely to be strong determining factors in the choice of assay to assess thiamine status.

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**Table 1:** Selected performance and use characteristics and minimal usage requirements for recommended assay methods for measuring human thiamine status in blood.

| Performance and usage characteristics                                                                                                                                                                                                                                                                                                                                                                                       | HPLC                                                                                                                                                         | LC-MS/MS                                                                                                                               | ETK activity assay                                                                                                                                                                                                                                                           |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>1. Assessment tool format and operator</b><br><br><i>Minimal criteria:</i> Tool is operable by a laboratorian with specialist training.<br><br><i>Optimal criteria:</i> Tool is a benchtop analyzer that can be operated by a laboratorian with basic training on a laptop computer.                                                                                                                                     | HPLC and fluorescence detector required in temperature-controlled lab. Instrument software required and substantial training                                 | Instrumentation requires a temperature-controlled environment, electricity, gases, and a vacuum pump and trained laboratory technician | Requires a benchtop ELISA plate reader with integrated incubator, associated software and a trained laboratory technician. Not all plate readers are suitable due to uneven heating across the plate. A procedure to check plate reader suitability has been published (18). |
| <b>2. Software requirements</b> for assay data reduction and results management<br><br><i>Minimal criteria:</i> Results can be generated for multiple samples at a time, and the software does not have data security restrictions that would otherwise challenge exporting results in batch.<br><br><i>Optimal criteria:</i> As a minimum, and additionally, the software does not require the user to purchase a license. | HPLC software is required (often with a user license) to run samples and process data.                                                                       | Software for instrument operation and data processing is required. A user license may be necessary.                                    | Standard ELISA plate reader software is required to run the assay. All calculations can be performed with Microsoft Excel or other spreadsheet software                                                                                                                      |
| <b>3. Reagent stability</b><br><br><i>Minimal criteria:</i> Long-term (6 months) storage, requiring refrigeration<br><br><i>Optimal criteria:</i> No refrigeration                                                                                                                                                                                                                                                          | Reagents require refrigeration and calibrator storage at -80°C. A full assay kit can be purchased from Chromsystems                                          | Reagents must be refrigerated. Calibrators, prepared controls, and internal standard solutions may be lyophilized and frozen at -80°C. | Reagents require -20°C freezer                                                                                                                                                                                                                                               |
| <b>4. Internal quality control</b><br><br><i>Minimal criteria:</i> Standard curves fit better than 0.95; sample controls; positive/negative controls required to monitor the quality of the kit, including low and high controls; calibration performed using appropriate reference method <u>if available</u> (e.g., monoplex immunoassay, HPLC, mass spectrometry).<br><br><i>Optimal criteria:</i> Same as minimum       | In-house controls prepared from donor materials or Chromsystems controls                                                                                     | In-house controls prepared from donor materials or Chromsystems controls                                                               | In-house controls prepared from whole blood                                                                                                                                                                                                                                  |
| <b>a. External quality control</b><br><br><i>Minimal criteria:</i> Values pass performance targets set by external performance verification scheme or values for reference material are within 10% of target values.<br><br><i>Optimal criteria:</i> Same as minimum                                                                                                                                                        | INSTAND EQA scheme and RCPA quality assurance program have ThDP in whole blood. No standard reference material in whole blood is available                   | External proficiency testing is available from providers such as INSTAND and RCPA.                                                     | No external quality control, quality assessment scheme, or reference materials                                                                                                                                                                                               |
| <b>b. Specimen type</b><br><br><i>Minimal criteria:</i><br>Venipuncture<br><br><i>Optimal criteria:</i><br>Capillary collection                                                                                                                                                                                                                                                                                             | Whole blood or red blood cells                                                                                                                               | Whole blood or red blood cells                                                                                                         | Standard assay for which status cut-offs exist requires washed red blood cells. Whole blood and dried blood spots can be used but associated cut-offs have not been defined and agreement between sample types has not been established.                                     |
| <b>5. Analytical specificity</b><br><br><i>Minimal criteria:</i> 95% (tested compounds should not give a response of more than 5% of the value expected for the analyte of interest).<br><br><i>Optimal criteria:</i> Same as minimum                                                                                                                                                                                       | Good chromatographic separation of thiamine, ThMP, and ThDP. Well-maintained equipment allows confidence in analytes based on retention time and peak shape. | Good chromatographic separation of thiamine, ThMP and ThDP. Chromatographic elution without ion suppression. Good mass resolution.     | Enzyme activity specific to thiamine-dependent transketolase                                                                                                                                                                                                                 |

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| <b>6. Precision (intra-assay variability)</b><br><br><i>Minimal criteria:</i> CV <10%<br><br><i>Optimal criteria:</i> Same as minimum                                                                                                                                                                                                                                                                                                                                                                        | <3% for ThDP, <2% for thiamine                                                                                                                                                                                                                                                                                                                                                                                                                                                             | <3%CV ThDP                                                                                                                                                               | Duplicate imprecision should not exceed 10%                                                                                              |
| <b>7. Reproducibility (inter-assay variability)</b><br><i>Minimal criteria:</i> CV < 15%<br>CV <25% at LLOQ <sup>2</sup> , ULOQ <sup>2</sup><br>LOQ and reportable range are reported.<br><br><i>Optimal criteria:</i> CV < 10%<br>LOQ and reportable range are reported.                                                                                                                                                                                                                                    | ThDP and thiamine LOQ 2 nmol/L. Inter-assay variability: ThDP <8.5%; Thiamine <15%. Reporting range: ThDP 2 – 400 nmol/L; thiamine 2 – 20 nmol/L                                                                                                                                                                                                                                                                                                                                           | ThDP <10% if >150 nmol/L; +/-15 nmol/L <150 nmol/L (RCPA criteria)                                                                                                       | When monitoring the ETKac ratios of QC material CV should be <5%                                                                         |
| <b>8. Linearity</b> (i.e., not of a standard curve, which is covered under internal QC, but of the analyte in the native matrix)<br><br><i>Minimal criteria:</i> r=0.95<br><br><i>Optimal criteria:</i> Same as minimum                                                                                                                                                                                                                                                                                      | Chromsystems lyophilysed whole blood L2 diluted with Chromsystems lyophilysed whole blood L1, r <sup>2</sup> >0.99                                                                                                                                                                                                                                                                                                                                                                         | Reporting range ThDP 20-1000 nmol/L                                                                                                                                      | N/A                                                                                                                                      |
| <b>Minimal usage requirements</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <b>HPLC</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | <b>LC-MS/MS</b>                                                                                                                                                          | <b>ETK activity assay</b>                                                                                                                |
| <b>1. Power and water requirements</b><br><br>Description: Does the instrument require uninterrupted daytime power or are power requirements minimal? Is access to de-ionized water required?                                                                                                                                                                                                                                                                                                                | Uninterrupted electric power. Deionized water is required for reagent preparation unless purchasing kits from Chromsystems where all reagents are supplied.                                                                                                                                                                                                                                                                                                                                | Uninterrupted electric power. Deionized water. Gas supply (tanked or generator).                                                                                         | The plate reader requires uninterrupted power when in use. Access to deionized water is required for the preparation of the TRIS buffer. |
| <b>2. Maintenance requirements and supply, services, and support</b><br><br>Description: (1) How frequently does the instrument require maintenance (in relative terms, i.e., as compared with other assays for the same biomarker) and what is the cost of parts (in relative terms)?<br><br>(2) What is the availability of servicing and support (i.e., can it be ordered and supplied directly by a manufacturer with global distribution, and is support offered via a network of global distributors)? | HPLCs require yearly preventive maintenance. ££ Servicing and support are available from suppliers and third-party companies.                                                                                                                                                                                                                                                                                                                                                              | Annual or semi-annual preventive maintenance. Service and as-needed repairs require trained service providers. ££££                                                      | Most plate readers will require a yearly service £ per year. Global distributors of plate readers are available,                         |
| <b>3. Waste management (hazardous materials/chemicals)</b><br><br>Description: What hazardous materials/chemicals are required? What waste management procedures are required as per WHO standards?                                                                                                                                                                                                                                                                                                          | Methanol; Toxic by skin contact and inhalation. Highly flammable<br>Orthophosphoric acid; Causes severe skin burns and eye damage.<br>Trichloroacetic Acid; Causes severe skin burns and eye damage. May cause respiratory irritation. Very Toxic to aquatic life.<br>Sodium Hydroxide; Corrosive, Exothermic reaction when added to water to create 15% solution. All should be disposed of by appropriate routes, by a waste management company that removes chemical and solvent waste. | Organic reagents and solvents, including methanol and acids (formic, hydrochloric, phosphoric, trichloroacetic). Approved waste management and disposal system required. | Hydrochloric Acid. Corrosive. Should be disposed of by appropriate routes.                                                               |
| <b>4. Ancillary equipment and supplies</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | HPLC column: Agilent Poroshell 120 EC-C18 3.0 x 50 mm, 2.7 µm                                                                                                                                                                                                                                                                                                                                                                                                                              | Labware: Centrifuge, shaker, vortex mixer, pipettes and tips.                                                                                                            | Multiskan™ FC microplate photometer with incubator                                                                                       |

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| <p>Description: Determined by the method: e.g. plate washer, pipettes and tips, plate reader, and ancillary supplies necessary for the performance of a lab-based immunoassay.</p> <p>Here, the fact that the methods derivatization in fluorescent thiochrome diphosphate derivative is not mentioned. This further requires sodium hydroxide and potassium ferricyanide as well as fluorescence spectrophotometer (more expensive than a spectrophotometer). It must be fluorescence spectrophotometer and not a more simpler filter fluorometer which lacks selectivity.</p> | <p>Centrifuge capable of being maintained at 4°C</p> <p>Agilent Captiva 96 well lipid removal plates</p> <p>2 mL polypropylene 96 well plates</p> <p>12 x 75 mm borosilicate glass tubes</p> <p>12 mL polypropylene tubes</p> <p>2 mL cryovials</p> <p>Analytical balance PH meter</p> <p>Vortex mixer Multitube vortex mixer</p> <p>200 µL Adjustable Pipette with tips</p> <p>1 mL Adjustable Pipette with filter tips</p> <p>5 mL Adjustable Pipette with tips</p> <p>Eppendorf repeating Pipette with tips</p> <p>Volumetric flasks and measuring cylinders of various sizes</p> <p>Positive pressure processor or vacuum pump for 96 well plates</p> <p>Fluorescence spectrophotometer</p> | <p>Refrigerator, freezer.</p> <p>Supplies: Analytical column, 96-well plates and sealing mats, glass vials and caps</p> | <p>Orbital microplate shaker with digital control</p> <p>Incubator with fan</p> <p>Refrigerated centrifuge</p> <p>Greiner UV-Star 96 well plates</p> <p>Microplate sealers</p> <p>Positive displacement pipette (50 µl – 200 µl) with disposable tips</p> <p>P20, P200, P1000 and P5000 Pipettes with tips</p> <p>1 ml Pastettes</p> <p>Sarstedt 2 ml microtubes</p> <p>Falcon 5 ml, 15 ml and 50 ml tubes</p> <p>Roller mixer</p> |
| <p><b>5. End-user price</b></p> <p>Description: In relative terms, i.e., as compared with other analytical methods for the same biomarker):</p> <p>(1) What are the capital equipment/infrastructure costs?</p> <p>(2) What is the cost per assay (considering consumables as well as person time)?</p>                                                                                                                                                                                                                                                                         | <p>Capital: \$\$\$\$</p> <p>Per assay costs: \$\$</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | <p>Capital: \$\$\$\$\$\$\$\$\$\$</p> <p>Per assay costs: \$\$\$</p>                                                     | <p>Capital: \$</p> <p>Per assay cost: \$</p>                                                                                                                                                                                                                                                                                                                                                                                       |

**Footnote:** The ETK assay requires less expensive equipment (microplate reader and photometer) than other methods, a small sample volume, and does not require calibration, but is only available in a small number of laboratories. Although not a direct measure of ThDP, similar functional assays are used for other nutrients, e.g. the erythrocyte glutathione reductase activity coefficient is used to assess vitamin B2 status (riboflavin). Commonly used cut-offs are based on measurements in washed red blood cells. Constant temperature over the whole plate is required as is an experienced analyst. HPLC is more expensive, no suitable internal standards exist, chromatographic separation is required as is a larger sample volume (>300 microliters), and high-throughput is challenging. A more robust laboratory infrastructure is also required. LC-MS/MS provides advantages in sensitivity and specificity and isotopically-labeled internal standards are available, but it is very expensive and methods can be difficult to set up. External proficiency testing schemes are available (e.g. RCPA and INSTAND), the use of which should be widely encouraged.