against the temperature (temperature ramp and then plotted monitored continuously during the temperature to 95 °C at a slope of 0.2 °C/s. 10 s, and then increasing the temperature (55 °C for 10 s), and extension (72 °C for 1 cycle at 95 °C for 0 s, 45 °C for 10 s). The melting curve consisted of 1 cycle at 95 °C for 0 s, 20 °C/s), annealing followed by 60 cycles of denaturation (95 °C for 120 s, and modified at the 3' end by phosphorylation to block extension. The PCR conditions were as follows: initial denaturation at 95 °C for 120 s, followed by 60 cycles of denaturation (95 °C for 0 s, 20 °C/s), annealing (55 °C for 10 s), and extension (72 °C for 10 s), and then increasing the temperature to 95 °C at a slope of 0.2 °C/s.

The fluorescence signal (F) was monitored continuously during the temperature ramp and then plotted against the temperature (T). These curves were transformed to derivative melting curves \((-\frac{dF}{dT})\) vs T).

Representative results for the three different genotypes (TT, CT, and CC) are given in Fig. 1. In the 100 patient samples, 27% were TT, 41% were CT, and 32% were CC. The proposed technique and the restriction enzyme technique gave identical results. The assay is rapid and accurate and seems especially suited for routine laboratories that process large numbers of samples.

We thank Olfert Landt (TIB MOL-BIOL, Tempelhofer Weg 11-12, 10829 Berlin, Germany) for designing the hybridization probes.

**References**


**Assessment of Vitamin B₁ Status**

To the Editor:

The excellent report by Talwar et al. (1) promotes the measurement of thiamin diphosphate (TDP) for the assessment of vitamin B₁ status. My experience with >30 000 people supports this, but only for the investigation of untreated patients.

The TDP assay is more precise than the transketolase activation (ETK) test, and the method described is an important advance for which I thank the authors. In comparing the two methods, Talwar et al. (1) found TDP slightly advantageous in the identification of B₁ deficiency. They and most workers using the ETK test agree that the cutoff point is 25%. I have found it useful to report results in the range 15–25% as borderline. When this is done, there is little to choose between TDP and ETK in terms of clinical usefulness.

Much as I would like to use the more precise TDP assay, there is a problem that surfaces when one wishes to use the laboratory to follow repletion with thiamin. It is very rare for the TDP concentration to remain low after a few days of supplemental B₁, and in many cases, TDP normalizes after a single 100-mg dose. This is not the case for the ETK test. In some cases, several weeks of daily supplementation are needed to normalize the results.

I am in the fortunate position of receiving considerable feedback from the clinicians using our laboratory service and have carefully studied their findings in relation to the laboratory results. In my experience, it is the ETK test that parallels the clinical improvement in supplemented patients.

I support the use of the more precise TDP (HPLC method) in untreated people, but I caution against its use in following supplementation. For this, the ETK test, even with its many limitations, remains the method of choice. A more precise method for measuring this enzyme would be enormously helpful.

I hope this letter will open some further discussion on the use of func-
Howard that further discussion is required on the merits or otherwise of direct and indirect measures of thiamin status in patients repelled with thiamin. Meanwhile, our experience with the HPLC assay suggests that measurement of TDP in red cells is the single most useful biochemical measurement for assessing thiamin status in patients who are at risk of thiamin deficiency.

References


Diners Talwar*
Department of Clinical Biochemistry
Macewen Building
Royal Infirmary
Glasgow G4 0SF, United Kingdom

*Author for correspondence.