

## References

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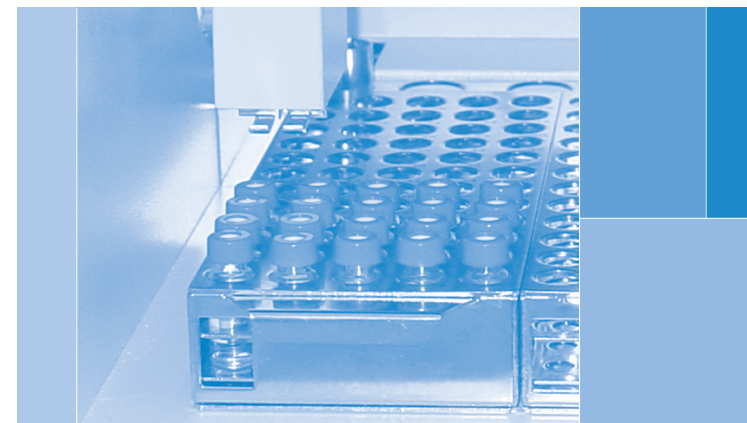
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## Thiopurine S-Methyltransferase Activity in Whole Blood TPMT [E.C. 2.1.1.67]



Vitamins and Gastroenterology  
Laboratory

Clinical Biochemistry  
City Hospital

Version No. 1.08

## Sending Specimens for Analysis

**Sample requirement:** A 2ml EDTA blood sample can be used for both TPMT phenotyping and genotyping analysis. Please note Lithium heparin samples can be used for phenotyping but are not suitable for genotyping.

- The sample **should not have been frozen**. If you have to store samples prior to dispatch please keep at 4°C.
- Please send samples by first class post at ambient temperature to the address on the back of this leaflet.

## Reference Ranges

Reference intervals for our whole blood TPMT phenotyping assay are as follows:

### Whole blood TPMT activity\* (nmol/g Hb/hour)

Deficient	≤ 5
Low	6 - 34
Normal	35 - 79
High	≥ 80

\* 37°C, pH 7.4 using 6-TG as substrate

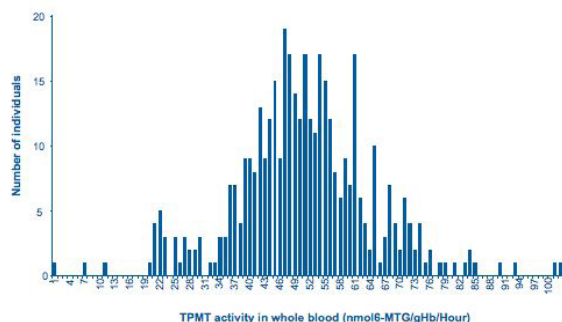


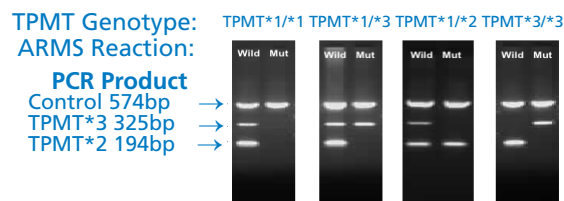
Figure: Distribution of TPMT activity for 400 whole blood patient samples

## City Hospital TPMT Service

Our whole blood phenotyping assay uses 6-thioguanine as the substrate and measures the product 6-methylthioguanine by HPLC<sup>1</sup>. The assay shows within batch and between batch imprecision of < 5%. We also use TPMT genotyping to confirm patient TPMT status for selected samples:

- Confirmation of deficient TPMT status
- Patients recently transfused (within 90 days)
- Patients that have had a previous severe reaction to thiopurine drugs
- Change in TPMT status on repeat testing

We use a multiplex amplification refractory mutation system (ARMS) strategy to screen for the common TPMT mutations TPMT\*2 and TPMT\*3, which account for approximately 95% of mutant TPMT alleles in most populations<sup>2</sup>.



Figure; Example results for TPMT genotyping

## Patient Information

Patients should be informed about the TPMT test prior to taking the sample and advised DNA confirmation may also be performed. The only known health implication for the genetic variation in TPMT expression is intolerance to thiopurine drugs. Patients can be directed to the website [www.labtestsonline.org.uk](http://www.labtestsonline.org.uk) for further information.

## Non-Genetic Factors Affecting TPMT Activity

- Samples from patients who have received a recent transfusion could give misleading results. Please note if informed we will perform genotyping to confirm patients TPMT status.
- TPMT is relatively stable and we have shown that at room temperature loss of activity is minimal for at least 8 days.

## Clinical Use of TPMT

Thiopurine drugs are widely used as immunosuppressants in gastroenterology and dermatology, in the treatment of acute lymphoblastic leukaemia, rheumatoid arthritis and post-transplant. These drugs are catabolised to inactive metabolites by the cytosolic enzyme, TPMT Thiopurine S-methyltransferase (TPMT)<sup>3</sup>.

TPMT activity exhibits autosomal co-dominant polymorphism. In a Caucasian population approximately 89% have normal enzyme activity, 11% low activity and 0.3% undetectable levels (deficient). Patients with undetectable TPMT activity are at most risk of developing severe reactions to a standard dose of thiopurine drugs.

Advances in understanding of the metabolism of thiopurines have led to significant changes in prescribing practice and toxicity monitoring<sup>4</sup>. The review by Alex Anstey<sup>5</sup> includes evidence based recommendations for routine monitoring of patients on azathioprine, including assessment of TPMT activity prior to treatment.