



Figure: Thiopurine Drug Metabolism.

[XO, Xanthine oxidase, HPRT, Hypoxanthine phosphoribosyltransferase, IMP inosine monophosphate and GMP, guanosine monophosphate].

References

1. Dubinsky M C et al. Pharmacogenomics and metabolite measurement for 6-mercaptopurine therapy in inflammatory bowel disease. *Gastroenterology* 2000; 118: 705-713.
2. Lennard L & Maddocks J. Assay of 6-thioguanine nucleotide, a major metabolite of azathioprine, 6-mercaptopurine and 6-thioguanine, in human red blood cells *J Pharm Pharmacol* 1983; 35: 15-18.
3. Dervieux T & Bouliou R. Simultaneous determination of 6-thioguanine and methyl 6-mercaptopurine nucleotides of azathioprine in red blood cells by HPLC. *Clin Chem* 1998; 1:156.
4. Maddocks J L et al. Azathioprine and severe bone marrow depression. *Lancet* 1986; 1:156.
5. Geary R B et al. Thiopurine methyltransferase and 6-thioguanine nucleotide measurement: early experience of use in clinical practice. *Intern Med J* 2005; 35:580-585.
6. Gardiner S J et al. Severe hepatotoxicity with high 6-methylmercaptopurine nucleotide concentrations after thiopurine dose escalation due to low 6-thiogaunine nucleotides. *Eur J Gastro Hep* 2008; 20:1238-1242.

Dr Jonathan Berg
 Head of Department
 Email: jonathanberg@nhs.net

Main Laboratory
 Tel: 0121 507 5345

Mailing Address
 Department of Clinical Biochemistry
 City Hospital
 Dudley Road
 Birmingham
 B18 7QH
 Fax: 0121 507 5290

www.cityassays.org.uk

 **CPA Accredited Laboratory**



Where
EVERYONE
 Matters



A Teaching Trust of The University of Birmingham
 Incorporating City, Sandwell and Rowley Regis Hospitals

© Sandwell and West Birmingham Hospitals NHS Trust

ML3466

Issue Date: August 2017
 Review Date: August 2020



Sandwell and West Birmingham Hospitals
 NHS Trust

Thiopurine Metabolites

Whole Blood
 6-Thioguanine Nucleotides (6TGN) and
 6-Methylmercaptopurine Nucleotides
 (6MMPN)

Clinical Biochemistry



Where
EVERYONE
 Matters



Sending Specimens for Analysis

- Sample requirement: minimum 0.5 mL EDTA whole blood.
- Samples must not have been frozen. Please store samples prior to dispatch at 4°C.
- Send samples by first class post at ambient temperature to the address on the back of this leaflet. We receive samples on a Saturday.
- Please provide details of current thiopurine drug regime and patient diagnosis on the request form.
- TPMT activity can also be undertaken on this sample but must be requested on the form.

Sample Timing

Thiopurine metabolites have a half-life of several days and so there is no need to take a sample at any special time. Steady state concentrations are reached between 2-4 weeks after a dose change. We suggest a sample for therapeutic drug monitoring is timed at 4 weeks from the start of treatment or a change in dose.

Sample Stability

In-house studies show that thiopurine metabolites are stable for at least 7 days at 4°C but for less than 3 days at room temperature. We highlight samples > 5 days old on receipt.

Interpretative Limits

235 – 450 pmol 6TGN/8x10⁸ cells
Maximum drug efficacy in inflammatory bowel disease.¹

>5700 pmol 6MMPN/8x10⁸ cells
Associated with increased risk of hepatotoxicity.¹

Assay Methodology

Acid hydrolysis liberates 6TGN and 6MMPN from red blood cells and converts them to 6-thioguanine and a 6-methylmercaptapurine derivative respectively. These compounds are then measured simultaneously by reverse-phase HPLC. The assay shows within-batch and between-batch imprecision of <8%.

Clinical Use of Thiopurine Metabolites

The immunosuppressive effect of thiopurine drugs is mediated primarily by the cytotoxic metabolite, 6TGN, and incorporation of these false bases into DNA. Accumulation of high levels of 6TGN is also responsible for some side effects of thiopurine drugs, and has been associated with leucopenia.⁴ Furthermore, high levels of the inactive metabolite 6MMPN, which is formed via the TPMT pathway, may be associated with hepatotoxicity.^{1,6}

Indications for Therapeutic Drug Monitoring of Thiopurine Metabolites

- Treating patients with low TPMT activity
- Suspected non-compliance or treatment with a suboptimal dose
- Failure to respond to standard doses of thiopurine drugs.^{5,6}

Low TPMT Activity

Patients with deficient or low thiopurine s-methyltransferase (TPMT) activity shunt 6-mercaptopurine towards increased 6TGN production. It is therefore strongly advised that patient TPMT status is tested prior to commencing thiopurine therapy.

Failure to Respond to Thiopurine Therapy

Measurement of 6MMPN helps to distinguish patients who are under-dosed or non-compliant (6MMPN levels appropriately low) from those demonstrating resistance to thiopurine drugs, i.e., preferentially metabolising thiopurine drugs to inactive 6MMPN rather than 6TGN (6MMPN disproportionately increased). In resistant patients, increasing the azathioprine dose is not helpful and further increases 6MMPN levels, predisposing to hepatotoxicity.⁶

