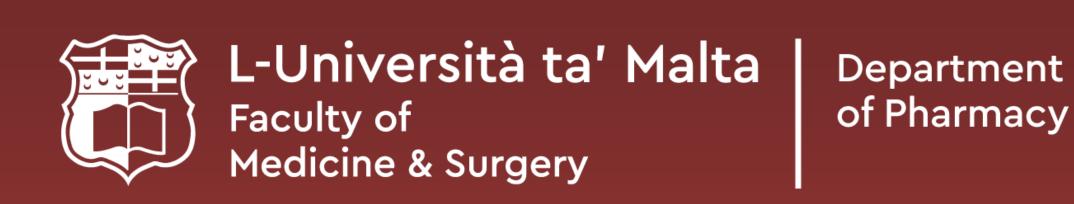
# Review of methods for TPMT biomarker genotyping in the personalisation of thiopurine therapy



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#### **INTRODUCTION**

Thiopurines, such as azathioprine, are used to treat leukaemias, Crohn's disease, rheumatoid arthritis, and as immunosuppressants after organ transplantation. Thiopurines are metabolised by thiopurine S-methyltransferase (TPMT) and impaired TPMT enzyme activity results in accumulation of thiopurines and toxic effects on bone marrow. TPMT-deficient patients are poor metabolisers and can be treated successfully and safely with reduced azathioprine doses if TPMT metaboliser status is identified by pharmacogenetic testing. Azathioprine has a 'Testing recommended' drug label annotation by the United States Food and Drug Administration.3

# AIM

To review and compare genotyping methods for the identification of the TPMT pharmacogenetic biomarker in relation to azathioprine therapy

#### **METHOD**

- Twenty-three research articles in the English language published between 1997
  and 2017 describing TPMT biomarker genotyping methods/assays in relation to
  azathioprine therapy were identified using PubMed, HyDi, ResearchGate and
  Google scholar and were reviewed.
- Product information of the genotyping assays was reviewed and company representatives were contacted when further information was required.
- Ten method characteristics were identified for comparative analysis of each genotyping method/assay (Table 1).

## RESULTS

- Twelve genotyping methods were identified; 10 Molecular and
   2 Biochemical-Analytical (Table 2).
- All 12 methods have high sensitivity and specificity (≥90%).
- Concordance with DNA sequencing (reference method) was 100% for all methods.
- The most common genomic DNA source is blood, however pyrosequencing allows use of biopsy samples and soft tissue samples.
- ARMS-PCR, Real-time PCR and the Strip assay have integrated controls in the test kits used.
- All methods except High Resolution Mutation Analysis have high reproducibility and all methods are reported to have high resolution and robustness.
- Time for analysis ranges from 0.33 to 9 hours and estimated cost per test ranges
   from €3 to €90 (Table 2).

#### Table 1. Characteristics evaluated for genotyping methods/assays

Sensitivity	Specificity  e Presence of Integrated Controls	
Genomic DNA source		
Reproducibility	Resolution	
Concordance	Robustness	
Estimated cost per test	Time for genotyping	

Table 2. Comparison of time for analysis and cost of TPMT genotyping methods reviewed

Tes	st method	Time for analysis (Hours)	Estimated cost/test (€)
	High Resolution	2	Not
	Mutation Analysis		reported
	Pyrosequencing	3.5	9
	Reverse	1	15
Molecular	Hybridisation Probe		
	DNA Microchip	3	90
	Technology		
	Single-strand	9	
	Conformation		Not
	Polymorphism		reported
	Southern Blot	4	•
	Hybridisation		
	Amplification	4	3
	Refractory Mutation		
	System (ARMS)-PCR		
	Restriction	3	3.5
	Fragment Length		
	Polymorphism-PCR		
	Real-Time PCR	1.17	45
	Strip Assay	6	_
Biochemical- Analytical	Denaturing High	0.33	Not
	Performance Liquid		reported
	Chromatography		_
	Mass	1	
	Spectrometry		

## CONCLUSION

This review demonstrates that for the methods considered, sensitivity, specificity, concordance, reproducibility, resolution, and robustness are high, and time and cost are variable. The possibility for non-invasive testing and presence of integrated controls are limited. The need for development of rapid, point-of-care genotyping methods necessary for timely implementation of pharmacogenetic testing in practice to improve patient safety is recognised.

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