

Microsampling-Based Quantification of Thiopurine Metabolites in Pediatric Solid Organ Transplant Recipients: LC-MS/MS Method Using VAMS, qDBS, and Conventional Blood Sampling

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INTRODUCTION

- Azathioprine (AZA) remains an important immunosuppressive agent in selected pediatric transplant recipients. Its metabolism is highly variable, and measurement of 6-thioguanine metabolites (6-TG) and 6-methylmercaptopurine metabolites (6-MMP) may support individualized therapy and assessment of adherence, efficacy, and toxicity (Figure 1).
- Conventional thiopurine therapeutic drug monitoring relies on venous blood collection and erythrocyte-based analysis. This approach can be burdensome in children and may limit the feasibility of frequent monitoring.
- Volumetric absorptive microsampling (VAMS) and quantitative dried blood spots (qDBS) require only 10 µL of blood and may provide a minimally invasive alternative suitable for decentralized TDM (Figure 2).

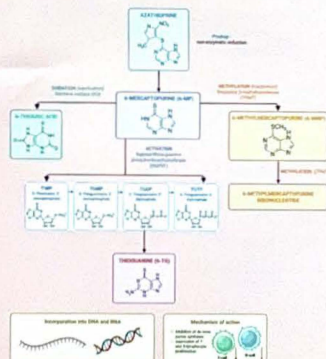


Figure 1. Azathioprine biotransformation to 6-mercaptopurine and downstream thiopurine metabolism. Created using BioRender.com under publishing rights.

METHODS

Aim: To develop and validate a rapid LC-MS/MS method for simultaneous quantification of 6-TG and 6-MMP in packed RBC, VAMS, and qDBS, and to assess its clinical applicability in pediatric renal transplant recipients receiving azathioprine.

- 8 pediatric kidney transplant recipients receiving azathioprine were prospectively enrolled.
- Each patient provided 5 paired sampling sets, resulting in: 40 samples per matrix
- Microsamples were collected simultaneously with conventional blood sampling, dried with desiccant, and subsequently analyzed.
- LC-MS/MS method parameters:**
 - Column:** Kinetex® EVO C18, 100 × 2.1 mm, 2.6 µm
 - Mobile phase:** water + 0.1% formic acid and acetonitrile + 0.1% formic acid
 - Mass spectrometer:** SCIEX QTRAP® 6500
 - MRM monitoring:** of 6-TG and 6-MMP (as hydrolysis derivative)

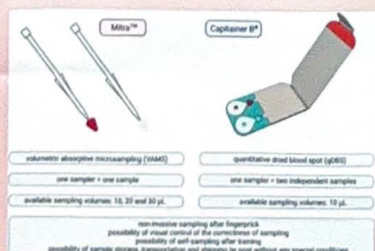


Figure 2. Comparison of evaluated microsampling devices in presented study - Capitainer® (Capitainer AB, Solna, Sweden) and Mitra™ (Neoteryx, Torrance, CA, USA)

RESULTS

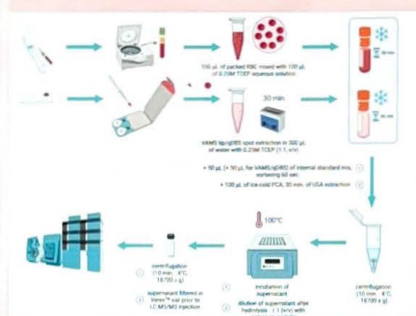


Figure 3. Schematic workflow of sample preparation for 6-TG and 6-MMP determination in RBC, VAMS, and qDBS samples. The workflow illustrates matrix-specific pretreatment followed by a unified hydrolysis and clean-up procedure prior to chromatographic analysis.

Parameter	Packed RBC	VAMS	qDBS
Calibration range (ng/mL)	6-TG: 10–3000 6-MMP: 50–3000	6-TG: 10–3000 6-MMP: 50–3000	6-TG: 10–3000 6-MMP: 50–3000
Linearity (r ²)	>0.998	>0.998	>0.998
LLOQ (ng/mL)	6-TG: 10 6-MMP: 50	6-TG: 10 6-MMP: 50	6-TG: 10 6-MMP: 50
Intra-day accuracy (bias, %)	−2.5 to 5.3	−3.0 to 6.2	−3.7 to 8.8
Intra-day precision (CV, %)	19.2	13.0	12.5
Inter-day accuracy (bias, %)	−3.0 to 6.4	−3.7 to 7.4	−4.3 to 11.0
Inter-day precision (CV, %)	13.0	13.8	13.8
Recovery (%)	6-TG: 80–90.0 6-MMP: 87.0	6-TG: 87.0–87.6 6-MMP: 83.3–84.9	6-TG: 84.0–85.0 6-MMP: 80.3–82.0
IS-normalized MF	0.98–1.02	0.98–1.02	0.97–1.02
MF variability (CV, %)	14.5	15.1	15.9
Hematocrit effect	Not applicable	Within ±15%	Within ±15%
Stability	Acceptable	Acceptable	Acceptable (within 6-TG at 30°C)

All validation results met ICH M10 acceptance criteria.

Figure 4. Results of method validations in three matrices - red blood cells (RBC), VAMS and qDBS samples.

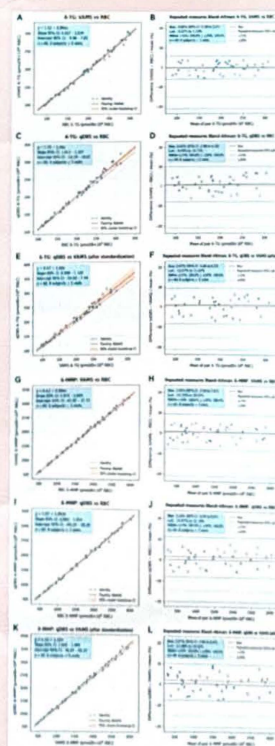


Figure 5. Head-to-head comparison of thiopurine metabolite concentrations obtained using VAMS, qDBS, and conventional packed RBC samples. Passing-Bablok regression plots (left) and repeated-measures Bland-Altman analyses (right) are shown for 6-TG and 6-MMP. Comparisons include VAMS vs packed RBC, qDBS vs packed RBC, and qDBS vs VAMS after erythrocyte-count standardization. Each analysis included 40 paired measurements from 8 pediatric renal transplant recipients (5 visits per patient). Dashed lines indicate identity or limits of agreement, while dotted lines denote predefined ±15% and ±20% agreement limits.

RESULTS

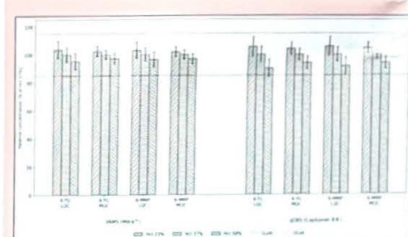


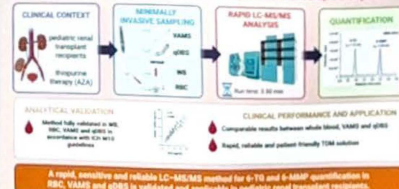
Figure 6. Evaluation of hematocrit (Hct) effects on the quantification of 6-thioguanine (6-TG) and 6-methylmercaptopurine (6-MMP) in volumetric absorptive microsampling (VAMS, Mitra™) and quantitative dried blood spot (qDBS, Capitainer B®) devices. Samples were prepared at low (LQC) and medium (MQC) quality-control concentrations using blood adjusted to hematocrit levels of 25%, 37%, and 58%. Results are expressed as relative concentrations (%) compared with the reference hematocrit level of 37%. Error bars represent standard deviation from five replicate measurements.

CONCLUSION

- VAMS and qDBS provide analytically reliable microsampling alternatives for thiopurine metabolite quantification in pediatric transplant recipients.
- Both microsampling approaches showed:
 - ✓ robust LC-MS/MS performance
 - ✓ limited hematocrit-related bias
 - ✓ good agreement with packed RBC
 - ✓ suitability for 10 µL blood samples
- Larger clinical studies are required before full clinical interchangeability with conventional erythrocyte-based monitoring can be established.

Rapid LC-MS/MS quantification of thiopurine metabolites (6-thioguanine and 6-methylmercaptopurine) in RBC, VAMS, and qDBS:

from analytical validation to clinical application in pediatric renal transplant recipients



TAKE-HOME MESSAGES

**10 µL BLOOD IN TWO MICROSAMPLING DEVICES
ONE RAPID LC-MS/MS METHOD**

VAMS and qDBS can substantially reduce the sampling burden of thiopurine TDM in children while maintaining close agreement with conventional RBC measurements.

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