development of a potential at-home assay for tacrolimus monitoring using a microsampling device

Stephen Stephenson[1], **Dr. James Rudge**[2]
[1]Leeds Teaching Hospital, St. James Hospital, Beckett Street, Leeds, West York LS9 7TF, England
[2]Neoteryx LLC, 421 Amapola Ave., Torrance, CA 90501 USA

Introduction

Tacrolimus is a member of the calcineurin inhibitor class of immunosuppressive drugs. Monitoring of these drugs is critical for maintaining vitality of a transplanted organ. Too high a dose can be cytotoxic and too little a dose can lead to tissue rejection[1]. Monitoring of these drugs often requires venous blood samples which can be problematic for some patients with collapsed veins and traumatic to vulnerable patients such as paediatrics. Furthermore, patients treated at home require regular monitoring which requires trips to a clinic which is disruptive to the patient and costly to the health service. Work outlined below uses the patent pending Mitra® Microsampler to collect 10 µL of blood which, after drying, was then extracted and analysed. These dried blood microsamples were then compared to wet blood samples from a standard analytical work-up. It is hoped that a less invasive assay can be developed compatible with at-home sampling through blood collection via a finger-prick at St. James' Hospital Leeds. On collection, samples would then be dried and then sent to a clinic via regular post.

Materials and Methods

In-House (Wet) Protein Precipitation Method

1. 25 µL of calibration standards/QC/ patient sample were placed in 1.5 mL Eppendorf® tubes
2. A solution of 100 µL 0.1 M zinc sulphate solution followed by 250 µL working IS solution (MeOH) was added to the samples
3. The tubes were capped and vortexed for 20 seconds then centrifuged for 5 minutes at 10,000 rpm
4. The supernatant was then analysed by 2D LC-MS/MS

Mitra Organic Extraction Method

1. Patient blood samples with calibrators (7) and QCS (4) were sampled with Mitra and dried for 1h
2. The Mitra tips were placed into a 96-well collection plate containing 200 µL of methanol (containing internal standard) and shaken for 1h (1100 rpm)
3. The tips were removed and the supernatant was dried then reconstituted in mobile phase
4. The samples were then analysed by 2D LC-MS/MS

Mitra Protein Precipitation Method

1. Patient blood samples with calibrators (7) and QCS (4) were sampled with Mitra and dried for 2h
2. The samplers were added to wells of a 96-well plate containing water (100 µL) and vortexed for ½ h after which the samplers were discarded
3. A premix of 100 µL of 8 % zinc sulfate and 100 µL of methanol with IS was added to each well and the plate was vortexed for 5 minutes
4. The samples were transferred to centrifuge tube (1.5 mL Eppendorf) and spun for 5 min
5. The samples were then analysed by 2D LC-MS/MS

Results and Discussion

The calibration curve shown in **Figure 1** showed that samples extracted from the Mitra tips showed excellent linearity and precision (R = 0.9993). The organic extraction method (**Figure 2**) showed a promising correlation compared to the in-house wed blood method (R^2 = 0.9034 but a large bias was observed). The organic extraction from the samplers were over reporting the in-house data (y = 0.5325). It was postulated that this was due to incomplete extraction of the calibrators and QCs compared to a better extraction of freshly dried patient samples. The calibrators and QCs were commercially available freeze-dried samples which were reconstituted in PBS before sampling. In-house work has shown that more stringent extraction conditions are often needed from aged blood compared to fresh. It was thus decided to mimic the in-house method as close as possible to try to reduced the a observed bias. The results from this (**Figure 3**) appeared to work very well, both linearity (R_2 = 0.9536) and accuracy (Y = 1.143) was significantly improved.

Figure 1. Calibration curve for tacrolimus from a Mitra Microsampler extraction

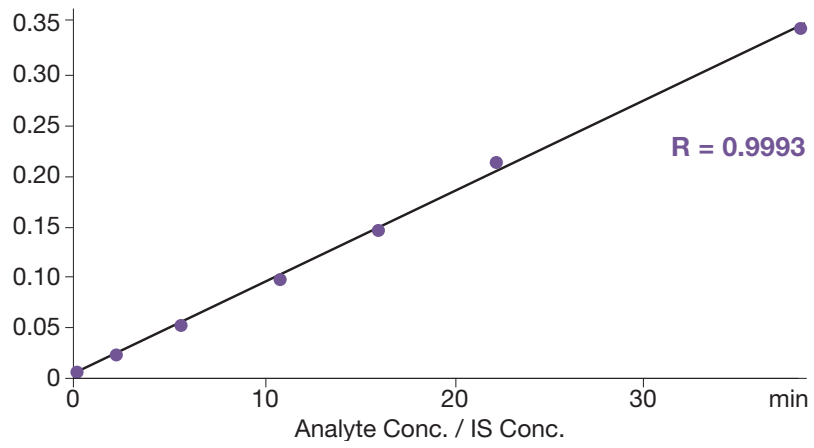


Figure 2. Comparing an organic extraction from Mitra Microsampler with standard wet preparation using patient samples

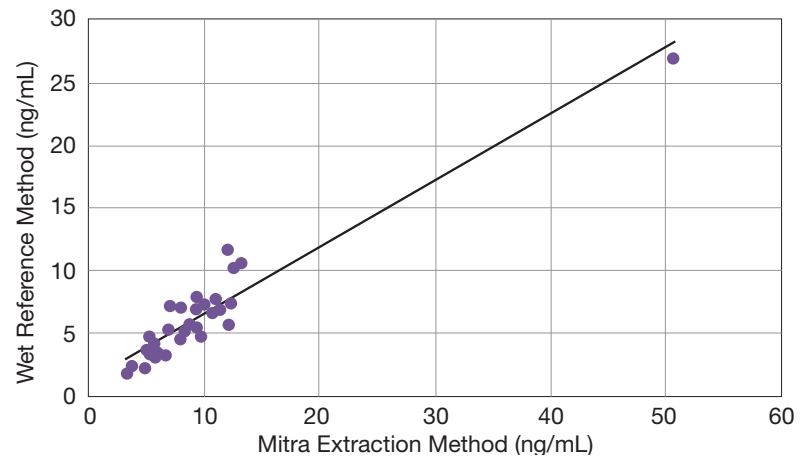
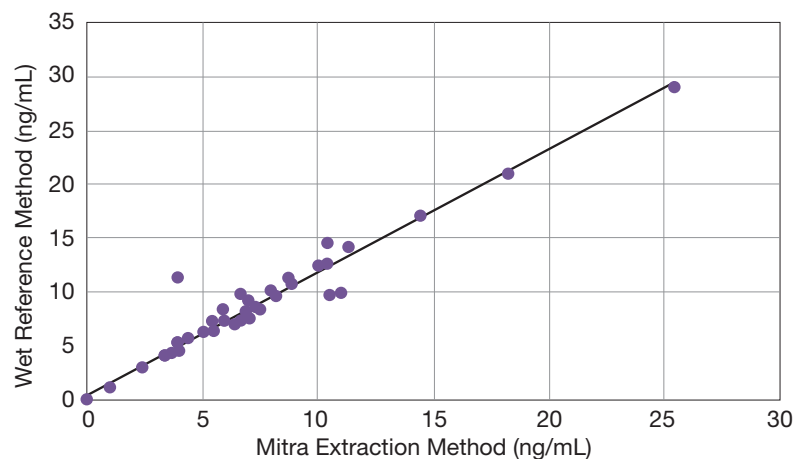


Figure 3. Comparing a protein precipitation extraction from Mitra Microsampler with standard wet preparation using patient samples



Conclusion

The study shows that there is a strong correlation between a dried Mitra Microsampling assay compared to wet lab assay for tacrolimus with a high level of precision and minimised assay bias. Assay bias could be further improved by honing the extraction conditions a little further. Alternatively, bias could be eliminated by conducting a bridging study using known wet blood samples. Both these possibilities and a longevity study are currently being investigated.

The work shows that an at-home therapeutic drug monitoring (TDM) method for tacrolimus has potential of making the patient experience less disruptive and reducing the cost to the health service.

References

1. Chisolm M, Lance CE, Mulloy LL. Patient Factors Associated With Adherence to Immunosuppressant Therapy in Renal Transplant Recipients. Am J Health Syst Pharm. 2005; 62 (17):1775-1781.

CE IVD

The Mitra Microsampler class I medical device is for direct specimen collection of blood and other biological fluids. It is not specific to any clinical test, and is not for use in diagnostic procedures. Use of the Mitra Microsampler in Laboratory Developed Tests (LDTs) requires further processing including the establishment of performance characteristics and successful validation by the laboratory in a manner consistent with CLIA requirements.

neoteryx®

The promise of microsampling, delivered

PO41760416_us