

development of a VAMS technique for IGF-1 in blood using microsampling for monitoring patients with acromegaly

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Introduction

Acromegaly is a condition which results in the development of abnormal soft tissue and skeletal growth¹. Prevalence of Acromegaly is 60 per million and there's a high mortality rate from sufferers². Acromegaly is caused by abnormally high serum levels of growth hormone (GH) due to over secretion by a tumour of the pituitary gland. When GH binds to GH receptors in liver and cartilage, Insulin-like Growth Factor-1 (IGF-1) is secreted. IGF-1 promotes cell proliferation, which causes abnormal bone muscle growth and apoptosis inhibition - resulting in acromegaly. Transsphenoidal surgery is the recommended primary therapy with biomarker post-operatively to measure any reoccurrence. GH secretion is pulsatile and varies widely over 24h whereas IGF-1 secretion is more continuous and so is a more stable biomarker in diagnosis and postoperative monitoring of the disease³. IGF-1 is usually measured in serum or plasma by immunoassay or, mass spectrometry in specialist labs. Immunoassay is simpler and faster but it is important to use an antibody with a high degree of specificity.

Currently, patients attend clinics to have their IGF-1 levels checked however, a far more convenient and inexpensive alternative would be to sample blood at home and post the sample to the lab for testing. Volumetric Absorptive Microsampling (VAMS[™]) offers such a solution. The Mitra[®] sampler (using VAMS technology) rapidly absorbs an accurate fixed volume of blood (10 or 20 µL) from a finger-prick (Figure 1) onto a hydrophilic porous polymer mounted onto a sampler body. The sampler body (in the shape of a pipette tip) is designed to be manually manipulated or automated in the laboratory for sample processing. Mitra samplers are housed in cartridges (Figure 2) which can be closed after the sampling event but allows air to circulate to dry the tips. When dry, many compounds are found to be stable in blood without the need for refrigeration.

The aim of this work was to assess if it would be possible to first detect IGF-1 in reconstituted blood samples from Mitra tips, to attempt to correlate this to wet serum or plasma and finally test the stability of the biomarker on the dried blood.

Figure 1. Sampling using Mitra device



Figure 2. Mitra clamshell cartridge format



Materials and Methods

Comparison of IGF-1 concentration in plasma and whole blood extracted with 10 µL Mitra tips

20 whole blood (EDTA) samples of IGF-1 concentration ranging from 50.8 to 515.4 ng/mL were measured to compare the IGF-1 results of the plasma and the whole blood extracted using VAMS. VAMS was used to sample the whole blood and air dried at room temperature for 2 hours. 2 VAMS was pooled into a micro container filled with 150 µl of distilled water and rotated on a rotating chamber for 1 hour. IGF-1 was measured using IDS-iSYS immunoassay analyser. The original whole blood was centrifuged and the plasma was measured directly on the analyser to compare the result with VAMS extracted whole blood.

Comparison of IGF-1 concentration of serum and whole blood extracted with 10 µL Mitra tips

10 paired whole blood (EDTA) and serum samples of IGF-1 concentration ranging from 96.9 to 515.4 ng/mL were measured to compare the IGF-1 results of the whole blood extracted using VAMS. Mitra tips were used to sample the whole blood and air dried in room temperature for 2 hours. 2 Mitra tips were pooled into micro container filled with 150 µl of distilled water and rotated on the rotating chamber for 1 hour. IGF-1 was measured using IDS-iSYS immunoassay analyser.

Stability experiment

16 VAMS sampled mid-level of IGF-1 of both serum and plasma of the same patient (serum IGF-1 of 160 ng/ml). Another 16 VAMS sampled high-level of IGF-1 of both serum and plasma of the same patient (serum IGF-1 of 577.5 ng/ml). The samples were dried in room temperature and extracted after 2 hr, 24 hr, 48 hr and 96 hr. The samples that were extracted earlier were stored in -20 °C freezer. IGF-1 level of all the samples were measured together in a single analytical run using IDS-iSYS immunoassay analyser.

Results and Discussion

Results from both the serum and plasma experiments demonstrated that IGF-1 was detected from reconstituted blood from the Mitra tips. The Mitra tips correlated very well when compared with both the plasma ($R^2 = 0.952$) and serum ($R^2 = 0.983$) results. Due to the fact that only 20 µL blood was taken per Mitra experiment allowed to dry and reconstituted in 150 µL, a dilution factor of 7.5 was applied to the data (Figures 3 and 4). Moreover, the data showed a positive bias for serum ($y = 0.7332$) and plasma ($y = 0.762$) when compared to VAMS-extracted whole blood. There are two possible reasons for this. The first is it is likely that IGF-1 is not present in whole blood cells so it would be expected to

see less IGF-1 in the same volume of blood when compared to serum or plasma. Indeed this is exactly what has been seen for Vitamin D extractions on Mitra[®]. Due to the fact that the Mitra extraction samples were 7.5 x diluted very low levels of IGF-1 were not detected (where the plasma concentration was below 85 ng/mL). This could be improved if more Mitra samplers were used in each extraction.

The stability experiment was very encouraging showing no significant loss (Figure 5) of IGF-1 even after 4 days dried at room temperature for both high (M) and low (K).

Figure 3. Comparison of IFG-1 result in plasma vs. VAMS extracted with whole blood.

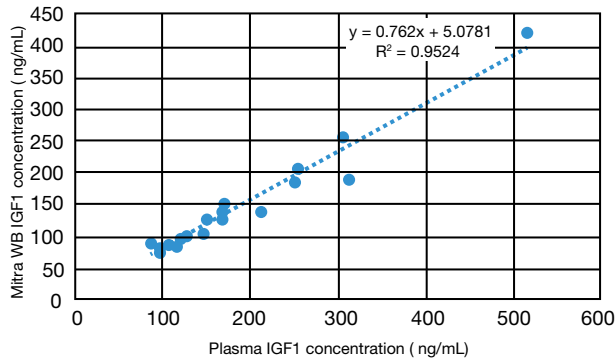


Figure 4. Comparison of IGF-1 result in serum vs. VAMS extracted with whole blood

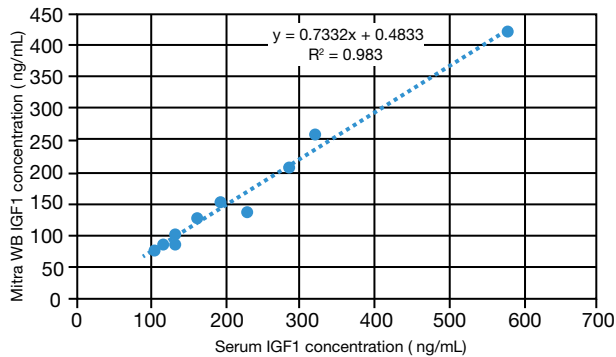
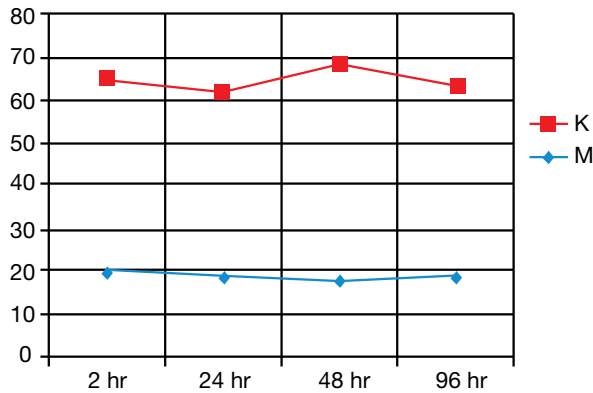


Figure 5. Stability of IGF-1 on dried Mitra microsamplers



Conclusion

The results shown in this investigation give very encouraging results that Mitra can be used for analysis of biomarkers on immunoassay instruments. Moreover, for IGF-1, dried-whole blood extracts from Mitra correlated very well with both wet plasma and serum samples. However, new reference ranges would be needed to be created due to the volumetric presence of the haematocrit. Furthermore, it was noted that the very

low level concentrations of IGF-1 were too dilute from Mitra and so efforts would be needed to reduce the amount of dilution or modifications would be needed to be done to the instrument to boost sensitivity. Nevertheless, the results show that as a convenient at-home monitoring option, Mitra microsampling devices demonstrate great promise.

References

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