WEQAS

Gas Chromatography Mass Spectrometry Reference Targets for the Comparison of Uric Acid Assays in Serum

UKAS CALIBRATION 4301

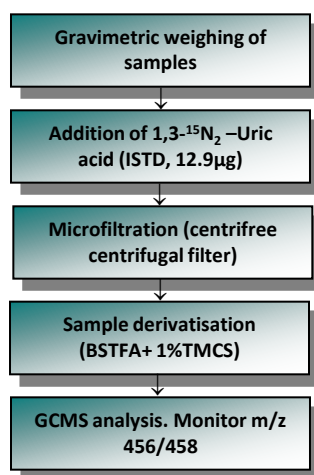Morton MS, Fraser H, Strevens C, Thomas MA, Ducroq DH

INTRODUCTION

EQA organisations such as WEQAS provide an essential role in the traceability of diagnostic products. All EQA providers will need to demonstrate traceability under the new ISO17043 standard. Traceability of results to the SI unit utilising reference target values is the preferred method of comparison of returned results where available, ensuring the transfer of accuracy from definitive methods to routine methods. Quality standards based on ISO15189 have now incorporated the need for laboratories to also demonstrate traceability. Use of reference targeted material would therefore assist in standardising results from these assays.

REFERENCE METHOD

An Isotope Dilution - Gas Chromatography Mass Spectrometry (ID-GCMS) method for Uric acid has been developed based on that of Thienpont[1]. The analysis was undertaken by the Reference Laboratory, a Division of the WEQAS Quality Laboratory. Samples were processed as detailed in the method flow diagram (fig. 1). Serum samples were equilibrated with a labelled $1,3-^{15}N_2$ analogue in a 1:1 ratio and then deproteinised by microfiltration. The microfiltrate was derivatised with BSTFA and 1% TMCS to form a tetra-TMS derivative. Traceability was assured by the use of NIST913a as a standard and NIST 909b levels I and II as a traceability control.
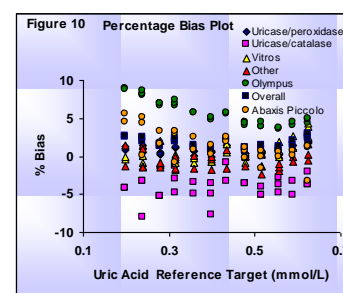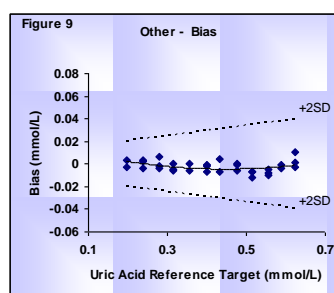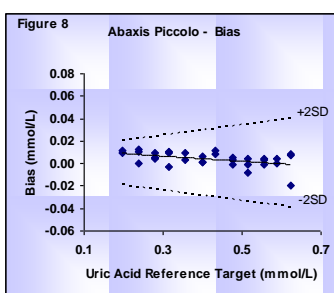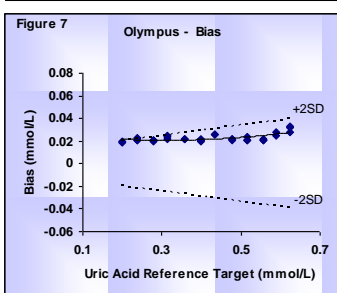
Figure 1
Method Flow Diagram

Gravimetric weighing of samples

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Addition of $1,3-^{15}N_2$ –Uric acid (ISTD, 12.9µg)

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Microfiltration (centrifree centrifugal filter)

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Sample derivatisation (BSTFA+ 1%TMCS)

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GCMS analysis. Monitor m/z 456/458

RESULTS

Target values for uric acid have been assigned to EQA material (0.2 - 0.7mmol/l analytical range) utilising Isotope Dilution 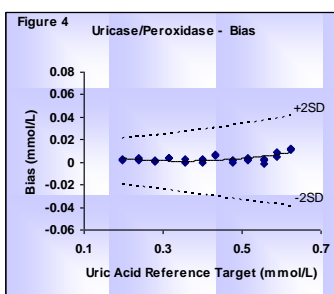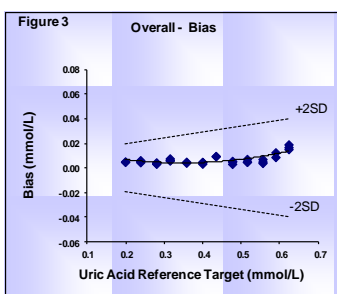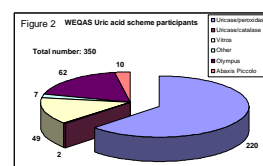Gas Chromatography Mass Spectrometry (ID-GCMS). The methodology was based on a published reference method. All twelve targeted pools (pools 711-722) were distributed over an eight month period.

All routine methods showed a polynomial regression fit compared to the reference target data (figs. 3-9). Figure 10 provides the percentage bias value across the range for each method group. The overall mean and uricase/peroxidase method group showed a similar pattern, as expected from the dominant group (fig. 2). Good agreement with the reference target was seen between 0.3-0.5mmol/L, with increasing bias (2%) evident above and below this level. The uricase/catalase method group showed an approximate 4% negative bias across the whole range. For the Vitros method, good agreement was observed up to 0.5mmol/L rising to a 4% bias above this level. The Olympus uricase/peroxidase method displays a different pattern to that observed for the overall uricase/peroxidase methods and all results were assigned to a separate group. Returned results for Olympus showed an approximately 9% positive bias at 0.2mmol/L dropping to 4% at 0.7mmol/L. The Abaxis Piccolo method group showed good agreement with the reference target values above 0.4mmol/L, rising to 5% positive bias below this level.

Figure 2 WEQAS Uric acid scheme participants
Total number: 350
Uricase/peroxidase, Uricase/catalase, Vitros, Other, Olympus, Abaxis Piccolo
10, 62, 7, 49, 2, 220

Figure 3 — Overall - Bias

Figure 4 — Uricase/Peroxidase - Bias

Figure 5 — Uricase/Catalase - Bias

Figure 6 — Vitros - Bias

Figure 7 — Olympus - Bias

Figure 8 — Abaxis Piccolo - Bias

Figure 9 — Other - Bias

Figure 10 — Percentage Bias Plot
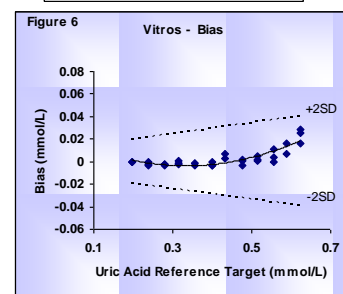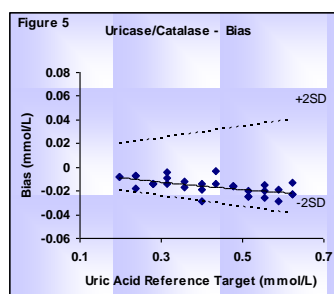
CONCLUSION

Comparison of results to an overall mean does not fully reflect the performance of individual methods. Reference method targeted data is clearly useful as an accuracy target in EQA Schemes. ID-GCMS values for uric acid will be assigned to all mainline chemistry pools allowing a comparison of participant results to the Reference method for each WEQAS distribution. The development of Reference methods will ensure that WEQAS can independently help manufacturers, users other EQA organisers and competent authorities in the post –marketing vigilance of the EU Directive 98/79/EC. WEQAS is committed to provide traceable reference method target values for the majority of analytes; currently the repertoire includes electrolytes, HbA1c, cholesterol, triglyceride, HDL cholesterol, steroids, bile acids, glucose, creatinine and enzymes.

REFERENCES

1. Thienpont L.M. Candidate Reference Methods for Determining Target Values for Cholesterol, Creatinine, Uric Acid, and Glucose in External Quality Assessment and Internal Accuracy Control. II. Method Transfer. Clin Chem 1993;39/6:1001-1006 .