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# ID-LC/MS-MS Reference Measurement Method for Cortisol in Serum

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## Introduction

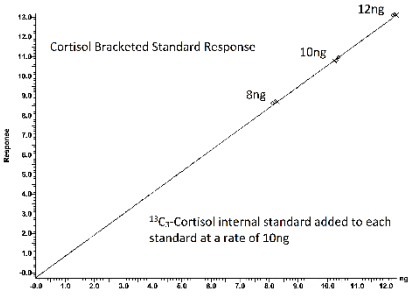
The traceability of laboratory results is a recognised need within quality standards based on ISO 15189 and ISO 17025. Where possible, higher order reference measurement methods are required to provide traceability to the SI unit for vitro diagnostic measurement results, ensuring the transfer of accuracy from definitive methods to routine methods.

A reference measurement method for cortisol based on a published method[1] using exact matching isotope dilution LC-MS/MS has been developed and validated within the Weqas Reference Measurement Laboratory. Traceability was assured by the use of NIST 921 (certified standard), with an accuracy matrix check using ERM-DA192 and ERM-DA193.

## Method

Gravimetric sample preparation used exact matching isotope dilution with a $^{13}C_3$-cortisol internal standard (10 ng of cortisol in samples and 10 ng internal standard). Cortisol was extracted from the serum matrix using Isolute® C18 solid phase extraction columns. Reconstituted samples were measured using a Waters Acquity UPLC I-Class coupled to a Waters Xevo TQ-XS triple quadrupole mass spectrometer, ionised by electrospray ionisation in positive mode. The column of choice was an XSelect HSS PFP (High Strength Silica Pentafluorophenol) 2.5 µm, 2.1 x 100 mm Column HP (Waters) with a mobile phase of ammonium acetate (2 mM) and 0.1% formic acid in (A) Water and (B) Methanol (60:40). The mass spectrometer was used in mixed reaction mode (MRM), monitoring ions m/z 363.3 (cortisol P), 97.15, 121.2 (cortisol D) and m/z 366.3 ($^{13}C_3$-cortisol P), 100.15, 124.2 ($^{13}C_3$-cortisol D) for each sample, control and standard. The ion pair ratio was converted to a cortisol concentration via reference to bracketed standard curves (fig. 1). Duplicate measurements of samples were made on three separate occasions. An MS scan was also carried out during analysis, to detect any potential contaminants.

**Figure 1** Cortisol Bracketed Standards




## Results

No major interferents were identified during the validation phase following a mass spectral scan of stock solutions at a concentration of 1 µg/mL (table 1). Where the observed masses were close to the monitored mass for cortisol, the retention time was observed to be greater than that for cortisol, aside from prednisolone.

Further investigation with addition of 10 ng prednisolone to cortisol stock standard material, measured against bracketed standards, showed no interference. Linearity was assessed by preparation of a series of samples in charcoal stripped serum ranging from 25 to 2000 nmol/L. (fig. 2). The correlation coefficient ($R^2$) of 1 and slope for the line as 1.007 indicate the method is linear across the measured range. The limit of detection (LOD) was assessed to be less than 0.5 nmol/L (fig. 3), although with exact matching isotope dilution, increased volume of sample could be used to increase response signal for lower concentration samples.

Bias of certified reference material was also within accepted criteria for a mass spectrometry method for cortisol (table 2). The maximum imprecision was within published reference measurement criteria based on duplicate analysis of samples on 6 separate occasions. Internally prepared IQC material prepared by spiking certified cortisol (NIST 921) into charcoal stripped serum also showed good reproducibility when measured in duplicate on 6 separate occasions.

Good performance of the method was observed within the IFCC RELA external quality assessment programme for Reference Measurement Laboratories. Data shown for RELA 2017 indicate measured values within the limits of equivalence (fig. 4) and comparable to other Reference Measurement Laboratories.

**Table 1** Interference Data

| Interfering compound | Retention time | Mass |
|---|---|---|
| Corticosterone | N/A | 346.47 |
| Cortisone | N/A | 360.45 |
| 11-Deoxycortisol | N/A | 346.47 |
| Dexamethasone | N/A | 392.461 |
| Progesterone | N/A | 314.46 |
| Prednisone | N/A | 358.428 |
| Prednisolone | RT 3.8/4.92 | 360.444 |
| 17α hydroxyprogesterone | N/A | 330.46 |
| Testosterone | N/A | 288.42 |
| 5α-pregnan-11ß, 17, 21-triol-3-20-dione | RT 3.71/4.92 | 364.48 |
| 5α-pregnan-3α, 11ß, 17, 21-tetrol-20-dione | RT 3.8/4.92 | 366.49 |
| 5ß-pregnan-3α, 11ß, 17, 21-tetrol-20-dione | N/A | 366.49 |
| 5α-pregnan-3ß, 11ß, 17, 21-tetrol-20-dione | N/A | 366.49 |
| 5ß-pregnan-11ß, 17, 21-triol-3-20-dione | 3.8/4.92 | 364.48 |
| Fenofibrate | N/A | 360.831 |
| Omeprazole sulphone | N/A | 345.4 |

**Figure 2** Cortisol Method Linearity

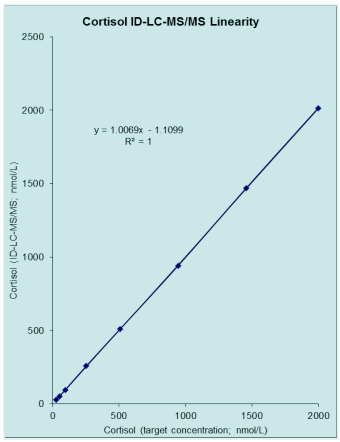



**Figure 3** Limit of Detection

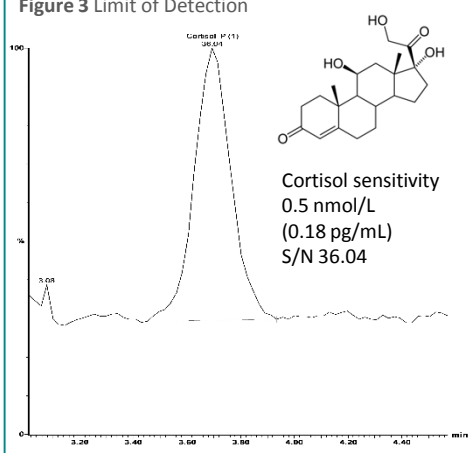
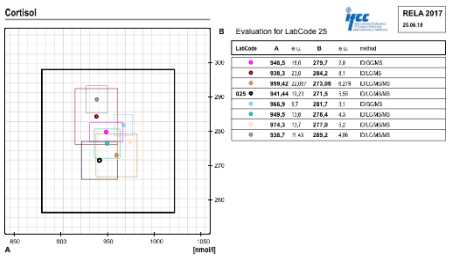



**Table 2** Cortisol Reference Material Traceability and Reproducibility Data

| Reference Material | Certified value (nmol/L) | Measured value (nmol/L) | N | % Bias | % CV |
|---|---|---|---|---|---|
| DA192 | 273 (267-279) | 274 | 12 | 0.80 | 1.37 |
| DA192 | 763 (749-777) | 757 | 12 | -0.25 | 1.07 |
| IQC-L | 292 (280-304) | 292 | 12 | -0.13 | 0.33 |
| IQC-H | 984 (944-1024) | 981 | 12 | -0.34 | 0.67 |

**Figure 4** RELA 2017 Performance




## Conclusions

A validated Reference Measurement Procedure has been developed and shown to be suitable for assigning values to human serum samples (EQA [PT], QC, standard and patient). This reference measurement method is being used to assign reference measurement values for all distributed samples within the Weqas Proficiency Testing Endocrine programme with an associated expanded uncertainty of 95%. The performance of routine cortisol methods can therefore be assessed relative to a gold standard method, ensuring traceability of field methods. Reference measurement data is known to be useful as an accuracy target in Proficiency Testing Programmes.

References

1. Hawley JM, Owen LJ, Mackenzie F, Mussell C, Cowen S, Keevil BG. Candidate reference measurement procedure for the quantification of total serum cortisol with LC-MS/MS. Clin Chem. 2016;62(1):262–9