Evaluation of Architect 2nd Generation Testosterone Assay Compared With Liquid Chromatography-Isotope Dilution Tandem Mass Spectrometry

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Introduction

Testosterone is an androgen hormone that plays a significant physiological role in men and it is also clinically important to be able to measure it accurately and precisely in women and children. Measurement is useful in the investigation of androgen-producing tumours, evaluation of response to anti-androgen therapy in prostate cancer, investigation of polycystic ovary syndrome in women and delayed puberty in children or ambiguous genitalia in neonates. Automated testosterone immunoassays have well known sensitivity and specificity problems particularly at the lower concentrations found in women and children. We compared the recently released Abbott Architect 2nd generation testosterone automated immunoassay (2Gtesto) with our routine liquid chromatography tandem mass spectrometry (LCMS/MS) assay.

Methods

Study Structure

339 samples consisting of 192 adult females, 89 adult males and 57 paediatric samples (23 females and 21 males) with ages ranging from 3 months to 16 years were assayed by both methods. Statistical analyses were performed using Microsoft Excel and Excel Analyse-It.

Abbott Architect

The Architect 2Gtesto assay is a direct one step chemiluminescent microparticle immunoassay (CMA). Assay specific clulant and anti-testosterone antibody (sheep, monoclonal) coated paramagnetic microparticles are combined. Testosterone present in the sample binds to the anti-testosterone coated microparticles. After incubation, an acridinium-labelled testosterone conjugate is added to the reaction mixture. After further incubation and washing, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured to determine testosterone concentration. The precision of the Architect 2Gtesto assay was determined using three levels of quality control (QC) material (Bio-Rad LiOuchek Immunoassay Plus Control). Duplicate QC samples were assayed daily over a period of five days and the coefficient of variation (CV) determined at each level. At 3.3 nmol/L, the CV was 0.33% and at 19.0 nmol/L, the CV was 1.04% and at 34.0 nmol/L, the CV was 0.41%.

Water Acquity UPLC

LCMS/MS analysis was carried out using Acquity ultra performance liquid chromatography (UPLC) coupled to a Quattro Premier triple quadrupole mass spectrometer (Waters) to identify and acquire relative abundance data using positive electrospray ionisation (ESI+). Extracted samples, serum calibrators and QC samples were injected onto a 1.7 μm BEH C18 column, and acquire relative abundance data using positive electrospray ionisation (ESI+). Extracted (UPLC) coupled to a Quattro Premier triple quadrupole mass spectrometer (Waters) to identify and acquire relative abundance data using positive electrospray ionisation (ESI+). Extracted samples, serum calibrators and QC samples were injected onto a 1.7 μm BEH C18 column, and acquire relative abundance data using positive electrospray ionisation (ESI+). Extracted samples, serum calibrators and QC samples were injected onto a 1.7 μm BEH C18 column, and acquire relative abundance data using positive electrospray ionisation (ESI+).

Results

Architect 2Gtesto Functional Sensitivity

Triplicate analyses of low concentration patient samples ranging from 0.1 to 6 nmol/L were conducted to determine the functional sensitivity of the assay, the concetration at which results can be determined with a CV of 20% (Figure 2). The results were consistent with the manufacturer’s claims of an LLOQ of ≤ 0.15 nmol/L.

LCMS/MS assay chromatograms displaying testosterone (quantifier) (m/z 289.3>96.7) and d2-testosterone internal standard (m/z 291.3 >110.7) transitions.

Sample comparison:

Comparison between the performance of Abbott 2Gtesto assay and the LCMS/MS assay for the 89 adult males (Figure 3) showed good correlation (R2 = 0.99) but with a small proportional bias (2Gtesto = 1.075 LCMS/MS + 0.3162). The results for the adult female samples (Figure 4) however showed less agreement between the two methods (R2 = 0.70, 2Gtesto = 0.9784 LCMS/MS + 0.3343). Notably several samples (10 of 160) gave discordant results relative to the respective assay reference intervals as highlighted in Figure 4. The results in all of these samples were above the upper limit of the Abbott expected values but were within the reference interval of the LCMS/MS assay. Results from paediatric females (Figure 5) demonstrated a similar trend to the adult female group (2Gtesto = 0.9538 LCMS/MS + 0.4762, R2 = 0.68) however fewer samples were available for the comparison. Good agreement (2Gtesto = 1.035 LCMS/MS + 0.033, R2 = 0.95) was demonstrated by paediatric males (Figure 6) but this comparison was also limited by the number of available samples.

Discussion

Measurement of low testosterone concentrations in paediatric and adult female samples is analytically challenging. The performance of commonly used automated testosterone immunoassays is limited in terms of analytical sensitivity and specificity. Our study results suggest that there is good agreement between the newly improved Abbott 2Gtesto assays with the LCMS/MS assay for adult males however there are still limitations, particularly with regard to the specificity, in its application in measuring testosterone in females and children. While there is reasonable agreement in the majority of female samples, there remains a significant number of samples from both adults and children in which there is possibly substantial cross-reactivity with other steroid hormones. In this study up to 3-fold increases were observed in results from the automated platform relative to those by the LCMS/MS assay. This limits the confidence that can be placed in any results greater than the reference interval.

Conclusion

The new automated Architect 2nd Generation Testosterone assay platform is capable of measuring male samples with acceptable accuracy and precision. However assay specificity remains a challenge for measuring testosterone in female adults and children.

References

2. Abbott 2nd Generation Testosterone, Kit inserts (IFU.)

LCMS vs Architect Testosterones in Adult Males

LCMS vs Architect Testosterones in Adult Males

LCMS vs Architect Testosterones in Adult Females

LCMS vs Architect Testosterones in Paediatric Females

LCMS vs Architect Testosterones in Adult Males

LCMS vs Architect Testosterones in Paediatric Males

Figure 1. LCMS/MS assay chromatograms displaying testosterone (quantifier) (m/z 289.3>96.7), testosterone (qualifier) (m/z 289.3>96.7) and d2-testosterone internal standard (m/z 291.3 >110.7) transitions.

Figure 2. Functional sensitivity curve for the Abbott Architect 2Gtesto assay, demonstrates that the assay performance is consistent with the manufacturer’s claim of a lower limit of quantification (LLOQ) of ≤ 0.15 nmol/L.