Harmonisation of Serum Dihydrotestosterone Analysis Establishment of an External Quality Assurance Program

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Background

Serum dihydrotestosterone (DHT) is an important analyte for the clinical assessment of disorders of sex development. It is also reportedly a difficult analyte to measure. Currently there are significant gaps in the standardisation of this analyte, including no external quality assurance (EQA) program available worldwide to allow for peer performance review of DHT.

We therefore proposed to establish an EQA program for serum DHT.

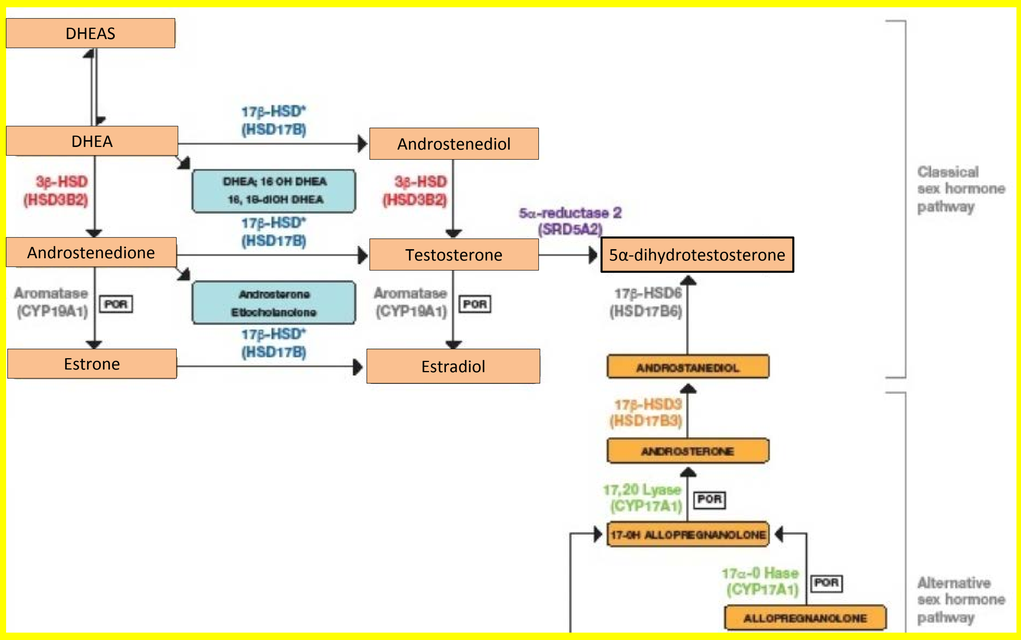


Figure 1: DHT is a C19 steroid which is the most biologically active androgen. It can be produced by the Classical or Alternative Sex Hormone Pathways .

Method

DHT was assessed in the 2015 Royal College of Pathologists of Australasia (RCPA) Quality Assurance Programs (QAP) Endocrine program material. The target (i.e. "true") values for the material were established using a measurement procedure based on isotope dilution GC-MS/MS. DHT calibrator values were based on weighed values of pure DHT material (>97.5% purity) from Sigma. The allowable limits of performance (ALP) were established as +/-0.1 up to 0.5 nmol/L and +/-15% for targets >0.5 nmol/L.

Conclusion

The DHT pilot ran successfully throughout 2015, and has now been formally included in the RCPAQAP Endocrine Program. Through the establishment of this EQA program, we now have the first peer comparison of serum DHT measurement by mass spectrometry and immunoassay laboratories [1]. This EQA program provides one of the pillars to achieve method harmonisation and eventual standardisation. This supports accurate clinical decisions where DHT measurement is required.

1. Greaves RF et al., Clinical Chemistry and Laboratory Medicine, 2016 in press

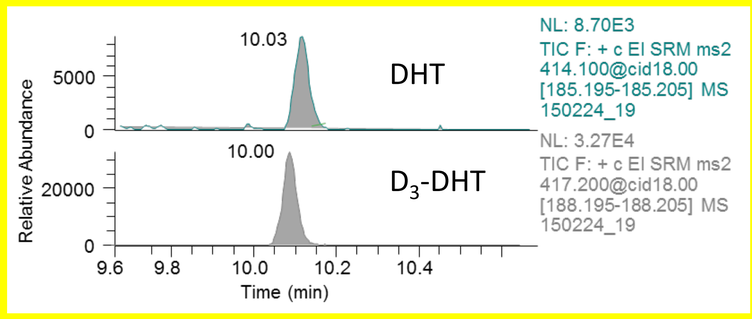


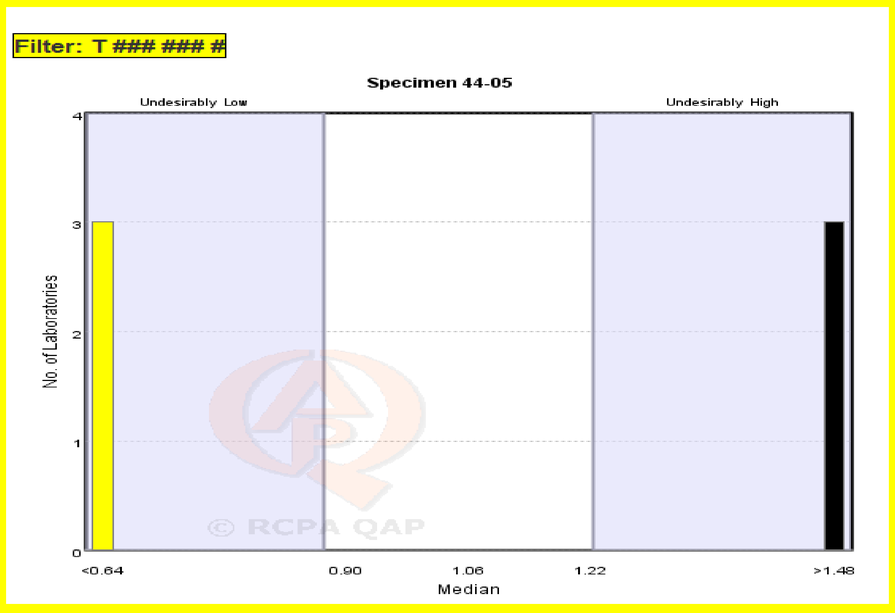
Figure 2: GC-MS/MS chromatogram of RCPAQAP material.

Results

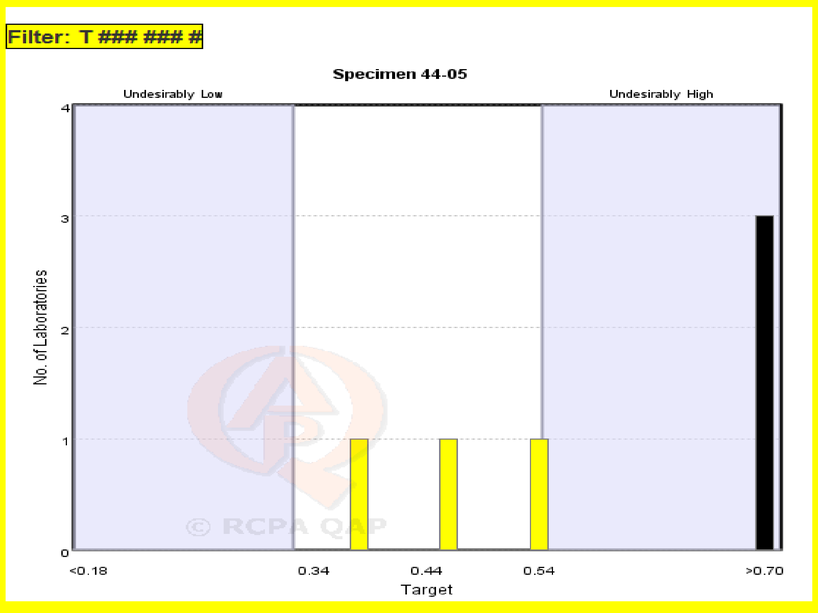
Target values for the six levels of RCPAQAP material for DHT ranged from 0.02 to 0.43 nmol/L (0.01 to 0.12 ng/mL). The material demonstrated linearity across the six levels with a best fit polynomial regression of y= 1.024 x + 0.002846. There were initially four participating laboratories at the start of pilot, which increased to seven by the end of the pilot study. Results of the LC-MS/MS methods were within the ALP when compared to the target values; whereas the results from the immunoassay methods were consistently higher than the target values and outside the ALP.

EN43 Level	1st Median nmol/L	No. of Results	2nd Median nmol/L	No. of Results	Target Value
1	0.52	2	0.27	4	0.02
2	0.73	3	0.45	4	0.11
3	0.38	4	0.25	5	0.19
4	0.86	4	0.31	5	0.28
5	0.64	3	0.4	5	0.36
6	0.87	4	0.6	5	0.44

Table 1: DHT Median results compared to established targets for Cycle 43



Level 6 Median



Level 6 Target

Figure 3: Level 6 results from immunoassay (black) and LC-MS/MS (yellow) based on Median compared to Target levels set by GC-MS/MS for Cycle 44 of Endocrine Program (second cycle of 2015). Results from 6 labs.

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