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Measurement of Free Testosterone in Serum using Equilibrium Dialysis Coupled with ID-UHPLC-MS/MS: in Comparison to Calculated FT

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Assessment of free testosterone (FT) has been recommended in recent clinical practice guidelines as a biomarker for diagnosis and management of testosterone related disorders such as male testosterone deficiency due to hypogonadism and female androgen excess due to polycystic ovary syndrome (PCOS). FT is currently measured using direct commercial immunoassays, assays using equilibrium dialysis (ED) or other separation techniques, or estimated by calculating free testosterone (cFT) using different formulas. The most widely used formula was developed by Vermeulen et al and uses total testosterone and sex hormone binding globulin (SHBG) concentrations. The ED approach is recognized as the gold standard; however, it is technically challenging for routine clinical use. CDC's Clinical Standardization Program (CDC CSP) has

developed a high throughput method using ED coupled with isotope dilution ultra-high-performance liquid chromatography tandem mass spectrometry. The study to evaluate the agreement of this ED-based method to the cFT using different algorithms is being conducted. Serum samples are dialyzed in a custom-designed multi-well plate against a protein-free HEPES buffer (pH 7.4) at 37 °C until equilibrium. After isolating endogenous FT from protein-bound testosterone by ED, isotope-labeled internal standard (13C3-testosterone) was added to the resulting dialysate for quantification. Certified pure primary reference material (National Measurement Institute-M914) was used to prepare calibrators, enabling traceable quantitation and ensuring measurement trueness. FT was further isolated from the dialysate matrix using supported liquid extraction and a chromatographic separation from interfering compounds and quantitation by tandem MS. The assay allowed detection of 2.9 pg/mL FT, with the bias within ± 5% and precision less than 10% CV. A total of 45 samples with a wide range of TT (21-912 ng/dL) and SHBG (11-129 nmol/L) were analyzed. The obtained FT values were compared to the cFT values obtained with the Vermeulen algorithm. The mean difference of 34.1% (95% CI 29.1% to 39.9%) between the measured and calculated FT were observed, with majority of the calculated values overestimating FT concentrations. The described high throughput method for FT allows for sufficiently accurate and precise measurement and can provide more reliable FT measurements compared to cFT (that can lead to overestimation of FT) for routine applications including large epidemiologic studies.

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