Reproductive Endocrinology
MALE REPRODUCTIVE HEALTH

Measurement of Free Testosterone in Serum Using Equilibrium DialysisCoupled With ID-UHPLC-MS/MS: Comparison Between Equilibrium Devices

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Free testosterone (FT) has been used as a biomarker in clinical patient care and public health research to assess and manage patients with androgenic abnormalities. The latest Endocrine Society clinical practice guideline for testosterone therapy in men with hypogonadism recommends measuring FT for those with borderline and low total testosterone concentrations, or those who have conditions that change SHBG concentrations, such as some metabolic or hormonal diseases, certain medication use, or SHBG genetic polymorphisms. Measuring FT is technically challenging and shows high variability. The CDC clinical standardization program is developing a high throughput method using the gold-standard equilibrium dialysis (ED) procedure with isotope dilution ultra-high-performance liquid chromatography tandem mass spectrometry (ID-UHPLC-MS/MS).
A serum sample was dialyzed 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 ($^{13}C_3$-testosterone) was added to the 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 dialysis step requires maintaining the endogenous free hormone equilibrium so that results in dialysate reflect FT concentrations in the blood without influence from dilution, temperature, or pH. The dialyzer system has a 1:1 sample-to-buffer volume and has been used in reference measurement procedures for free hormone measurements, serving as the standard for method performance comparison. Four commercially available devices designed for high throughput in a multiple well-plate format, requiring respective sample-to-buffer ratios, were evaluated for their recovery, speed, ease of automation by a liquid handling system, repeatability, and robustness. Preliminary results showed that a device with 1:1 sample-to-buffer volume had the most comparable results to those obtained from the standard dialyzer, with the mean bias less than 15%. The device with the highest sample-to-buffer ratio showed bias as high as 50%. These data suggest that controlling sample-to-buffer ratio is a critical step in ED FT method.

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Novel Oral Testosterone Formulation Improves Male Well Being Without Compromising International Prostate Symptom Scores

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Introduction: Male hypogonadism results from insufficient secretion of testosterone (T) and is characterized by low serum T concentrations. Common symptoms of hypogonadism include decreased libido, impotence, weakness, low energy, depression and/or loss of motivation, memory and concentrating issues, and sleep disturbances. Several forms of T replacement are available. Testosterone undecanoate (TU) is a testosterone prodrug available in oral formulations. A novel TU formulation, SOV2012-F1, has been submitted for FDA consideration under the name KYZATREX®. While TU efficacy is measured by serum total T, patientfocused endpoints such as Patient Reported Outcomes (PROs) are valuable indicators of well-being and psychosexual symptom abatement. **Methods:** A Phase 3, randomized, multicenter, open-label, active-controlled trial, comparing SOV2012F1 (testosterone undecanoate capsules) (n=214) with AndroGel® (1.62% topical testosterone gel) (n=100) enrolled males aged 18 to 65 years with hypogonadism (serum total T levels ≤281 ng/dL). A key exploratory endpoint was change from Baseline (ΔBL) after 52 weeks of treatment in the following PROs: International Prostate Symptom Score (IPSS), Psychosexual Daily Questionnaire (PDQ), Short Form Health Survey 36 item (SF-36), and the International Index of Erectile Function (IIEF). **Results:** Total or overall scores for all PROs (IPSS, PDQ, SF-36 and IIEF) showed increased improvement in the SOV2012-F1 group relative to the Androgel group, and all but IPSS demonstrated improvement relative to BL. For IPSS, due to the potential that T could worsen urinary symptoms, the ΔBL would ideally be small to reflect minimal impact. Change for the SOV2012-F1 and AndroGel groups was, respectively, 0.6 and 1.0. Further, the IPSS total score was not significantly different from BL in the patients receiving SOV20212-F1 ($p = 0.5659$). For PDQ, a clinically meaningful improvement of sexual desire in hypogonadal men age ≥65 years is ≥0.7; mean ΔBL was 1.6 in the SOV2012-F1 group versus 1.4 in the AndroGel group. In the SF-36, the mean ΔBL total score was 83.7 in the SOV2012-F1 group and 70.2 in the AndroGel group. Further, post hoc analysis of the Health Change category found a significant ($p \leq 0.05$) improvement in patient perspectives on health over the course of the study. The overall satisfaction score of the IIEF trended towards significance for the SOV2012-F1 group with a mean ΔBL score of 2.3 versus and 1.6 in the AndroGel group. The ΔBL for the 4 domains of male sexual function were small and consistent between the SOV2012-F1 and AndroGel groups. Comparable results were noted for Early Withdrawals and All Subjects across all PROs. **Conclusion:** Treatment with SOV2012-F1 for 52 weeks exceeded AndroGel patient satisfaction as measured by PROs including IPSS, PDQ, SF-36 and IIEF, demonstrating clinical distinction. Further analysis of SOV2012-F1 will be forthcoming.

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