Impact of an Enzymatic Inhibitor on Testosterone Levels in Human Plasma vs. Serum
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Purpose
Traditionally, testosterone measurement is performed in serum. However, it has been demonstrated that when testosterone undecanoate is taken orally, testosterone must be determined in plasma containing an enzyme inhibitor to prevent the hydrolysis of testosterone undecanoate. A positive difference of up to 20% was observed between the endogenous levels of testosterone in serum compared to plasma containing anticoagulant involving enzyme inhibitor. The purpose of this work was to evaluate different anticoagulants for the measurement of testosterone.

Methods
Testosterone is isolated from the biological matrix by automated LLE with MTBE. Chromatography is achieved using an ACE Excel 2 C18-PFP column and detection is performed with an API5000. Different collection tubes were tested: Serum, EDTA-NaF, NaF-K2C2O4, EDTA K2 and P800 (enzymatic inhibitor cocktail). Different proportions of NaF were also tested. Fresh whole blood from men donors was gathered to determine serum and plasma testosterone levels. Testosterone was determined in each plasma sample and compared to the testosterone level in serum from the same donor. Different donors were also tested.

Results
Testosterone was validated over the dynamic range of 60-12000 pg/mL in both human NaF/K2C2O4 plasma and serum. When different collection tubes were compared, it was observed that NaF introduces a bias of -18% on testosterone endogenous level compared to their corresponding serum tubes. When the percentage of NaF added was increased, the bias increased accordingly. EDTA K2 and P800 showed no difference vs. serum tubes. Testosterone was also determined in EDTA K2 and NaF/K2C2O4 whole blood. The testosterone level was similar in both matrices, showing that the bias is due to the partition plasma/RBC. Different donors were also evaluated and the bias was similar between each donor. EDTA-NaF collection tubes showed the lowest bias (-8.9%), due to their lower concentration of NaF (0.15%).

Conclusion
These experiments tend to show that NaF causes a negative bias to testosterone concentrations. This bias is due to the different partition plasma/RBC when NaF is used, compared to serum. EDTA-NaF anticoagulant presented less bias and was proven to be effective in preventing the hydrolysis of testosterone undecanoate.