A Rapid iMethod™ Test for Low Levels of Testosterone in Human Serum

iMethod™ Test for Low Testosterone Levels Version 1.0 for Cliquid® Software

The following information outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for research into the quantitation of low levels of Testosterone when using an AB SCIEX API 5000™ and AB SCIEX Triple Quad™ 5500 LC/MS/MS systems.

The method included is for the routine analysis of low levels of Testosterone in human serum. Calibration is performed using charcoal filtered serum samples of known concentration. Control samples at clinically relevant low and high concentrations are used to establish the calibration range. The method uses a deuterated Testosterone –D_3 analog as an internal standard.

The sample preparation is a simple extraction using a high purity hexane/ethyl acetate reagent, followed by injection onto a Phenomenex Luna 2.5 µm C18, 100 x 2 mm HPLC column, included with the method, connected to the Turbo V™ source of the mass spectrometer. The analytical run is performed with an LC run time of 5.5 minutes per sample using with a dynamic range of 20-5000 pg/mL.

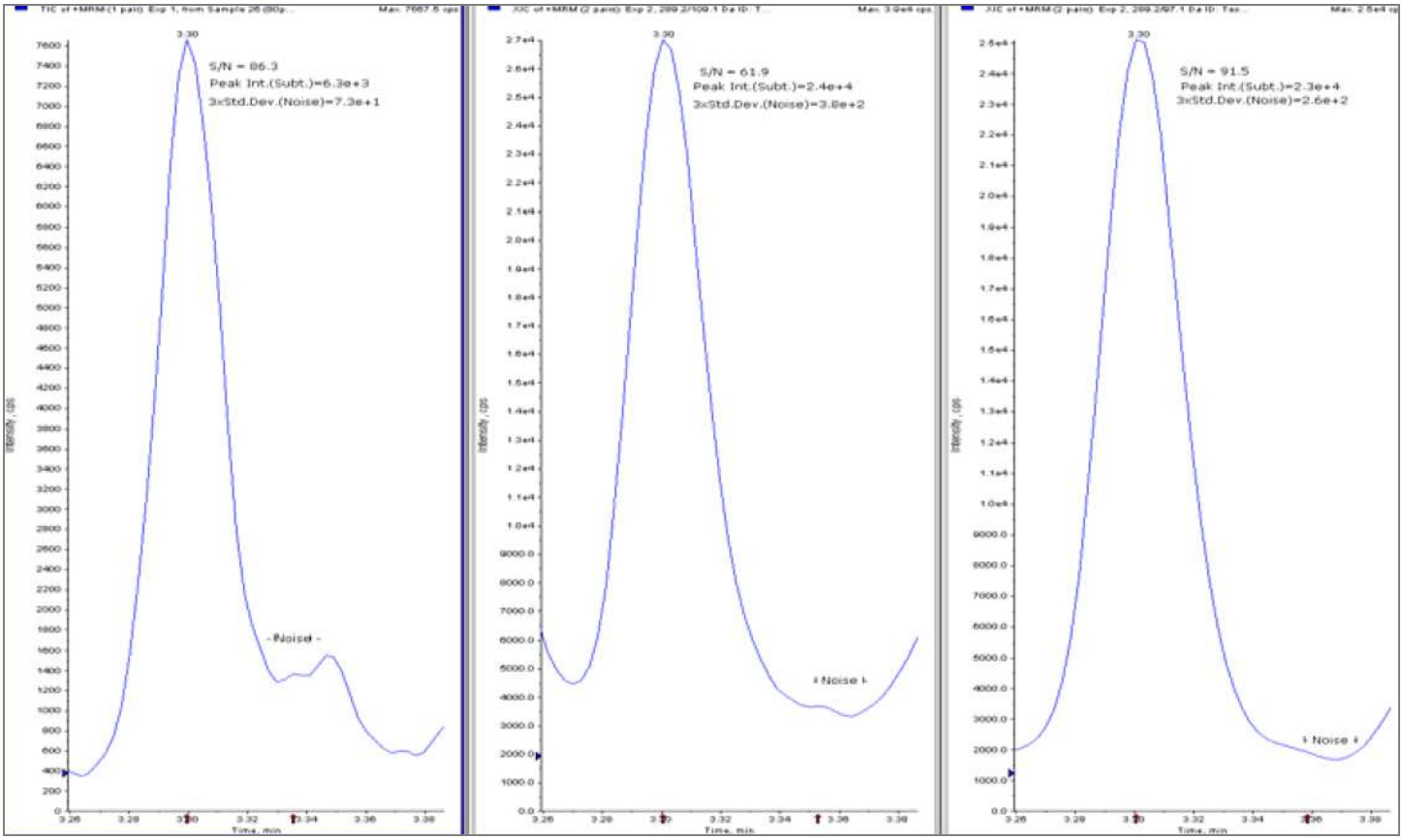

Figure 1: Example chromatograms of Testosterone at 80pg/mL with D_3-Testosterone (IS) run on an API 5000™ LC/MS/MS system

During the method evaluation, quantitation limits for each analyte were sufficient to allow the analytical method to be used for testosterone level quantitation. A typical intra-assay precision (n=4) for the low QC concentration is 10 %CV and an accuracy of 80% with a Signal to Noise (S/N) of 20. S/N is the peak height divided by the noise measured at 3 standard deviation of the noise.

Analyte (Transition)	STD Level (pg/mL)	Average %CV	Average S/N	LOD (pg/mL) @ S/N of 5
Testosterone-1 (289/97)	20	8	18	5.6
Testosterone-2 (289/109)	20	*10*	*15*	*6.7*

Calibration

The following calibration curves represent the linear dynamic range of from 20-5000 pg/mL for both the quantifier and qualifier transition, as expected for this assay.

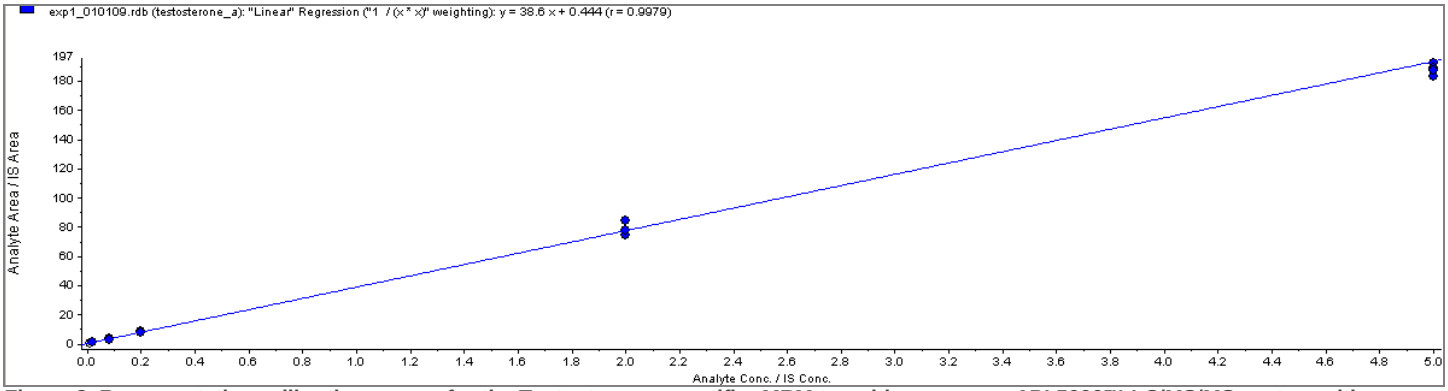

Figure 2. Representative calibration curves for the Testosterone quantifier MRM transition as on an API 5000™ LC/MS/MS system with a Shimadzu Prominence LC system during a 5 minute run with CV's (n=6) < 7% at the LLOQ (20 pg/ml) and an accuracy of within +/- 3% for all STD's and QC's.

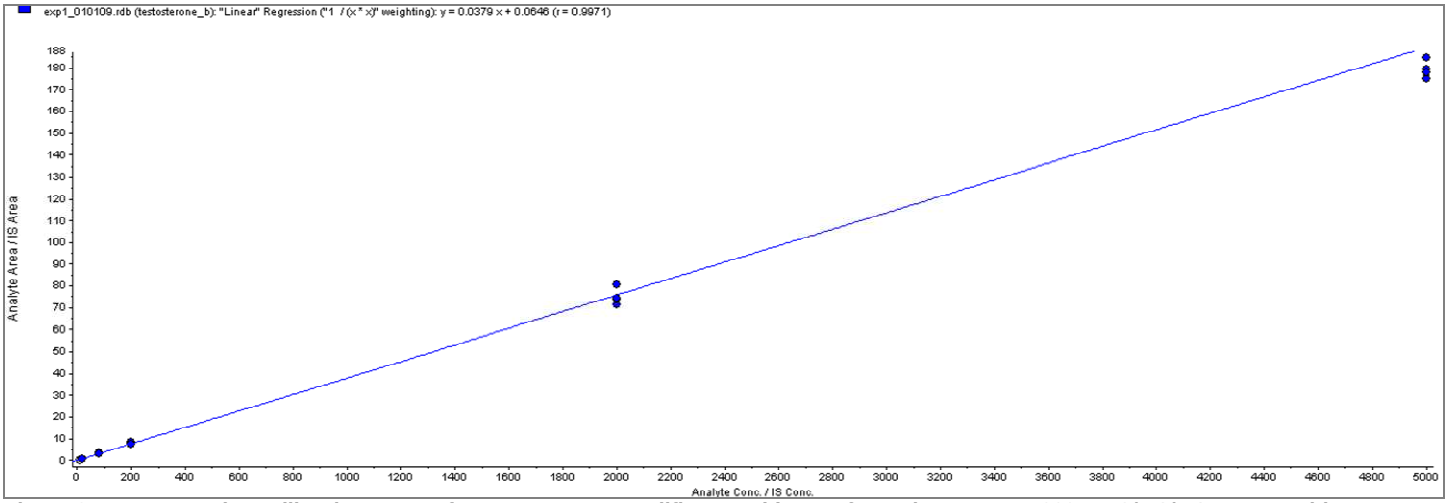

Figure 3. Representative calibration curves for Testosterone qualifier transition performed on an API 5000™ LC/MS/MS system with a Shimadzu Prominence LC system in a 5 minute run with CV's (n=6) for this experiment <7 % at the LLOQ (20 pg/mL). Accuracy was within +/- 3% for all STD's and QC's.

Please note that the results presented above were obtained using a single instrument and single set of standards and samples. Prior to production use, the method should be fully validated with real samples, and the results here may not be typical for all instruments. Variations in LC column properties, chemicals, environment, instrument performance and sample preparation procedures will impact performance, thus these results should be considered as informative rather than representative.

System Requirements

In order to run this method as outlined above, the following equipment and reagents are required:

- An AB SCIEX API 5000™ or AB SCIEX Triple Quad™ 5500 LC/MS/MS systems
- Shimadzu Prominence 20A HPLC system with reservoir tray and bottles, system controller CBM-20A, 100 µl mixer, 2 isocratic LC-20AD pumps, 3 channel degasser, SIL-20AC autosampler and column oven or an Agilent 1100/1200 HPLC system with binary pump, well plate autosampler and thermostated column oven.
- Charcoal filtered serum (www.goldenwestbio.com).
- Borosilicate centrifuge and sample handling tubes
- Testerosterone standard and deuterated internal standard (www.sigmaaldrich.com)
- HPLC grade hexane and ethyl acetate for Sample Preparation
- HPLC grade water, acetonitrile, methanol and formic acid
- 1.5 mL Eppendorf tubes
- A BetaBasic Javelin Guard Column 18, 5 µm 20 x 2.1 mm (Fisher Scientific)
- Phenomenex Luna 2.5 µm C18, 100 x 2 mm HPLC column (included)
- A centrifuge able to accommodate Eppendorf tubes and run at 14,000 rpm
- Pipettes and standard laboratory glassware

Ordering Information

Product Name	Part Number
iMethod™ Test for Low Testosterone Levels Version 1.0 for Cliquid® Software	1037084

While the information provided above outlines the instrument requirements and expected results obtainable from the AB SCIEX iMethod™ Test for Testosterones, please note that the results obtained do require some experience with LC/MS/MS and sample preparation procedures. As such, web-based and on-site training are available to assist in the deployment of the iMethod™ Test and are recommended for inexperienced users. Please consult your local sales representative for more details.

Important Note

The purchase and use of certain chemicals listed above may require the end user to possess any necessary licenses, permits or approvals, if such are required in accordance with local laws and regulations. It is the responsibility of the end user to purchase these chemicals from a licensed supplier, if required in accordance with local laws and regulations. The suppliers and part numbers listed below are for illustrative purposes only and may or may not meet the aforementioned local requirements.

The iMethod™ Test described above has been developed by AB SCIEX to provide all the sample prep and instrument parameters required to accelerate the adoption of this method for routine testing. The performance of this method will need to be verified in a given lab due to potential variations in instrument performance, maintenance, chemicals and procedures used, technical experience, sample matrices and environmental conditions It is the responsibility of the end user to make adjustments to this method to account for slight differences in equipment and/or materials from lab to lab as well as to determine and validate the performance of this method for a given instrument and sample type. Please note that a working knowledge of Analyst® Software may be required to do so.

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