Introduction
Steroid hormones are derived from cholesterol. Due to their fat-soluble characteristics these molecules can pass easily through the cell membrane and cause changes within the cell. Many endocrine diseases are caused by disorders in steroid metabolism. For diagnostic purposes, therefore, an accurate and high-throughput quantification of steroid hormones is of utmost importance.

Up till now immunoassays are lab standards but they often deliver unsatisfactory results due to inadequate antibody specificity. Moreover, a single parameter for each measurement leads to a low cost-efficiency when a wider panel of steroids is requested. These drawbacks can be successfully overcome with the application of SteroIDQ® Kit on a LC-MSMS platform. The 16 steroids included in this Kit are given below:

Principle
The SteroIDQ® Kit has been developed and validated for a LC-MS system consisting of a CTC PAL autosampler, an Agilent HPLC 1100/1200 binary pump, an Agilent column oven and an ABSciex 4000 / 4000QTRAP mass spectrometer with an ESI source. Other HPLC systems (for example from Shimadzu) have been also tested. Optionally the ABSciex 5500QTRAP mass spectrometer can be used to improved the sensitivity.

The following workflow and estimated time scale is applied for a full 96-well plate configuration (80 wells for samples, the rest for calibrators, blank and QC's). With smaller numbers of samples (1/2 plate for 36 samples or 1/3 plate for 20 samples) the measurement time (step 3) will be reduced accordingly.

1. Preparation of the HPLC-MS/MS System
   • Prepare mobile phases, wash solution, blank and Test mix,
   • Purge HPLC-MS/MS instrument, Condition HPLC column,
   • Perform System Suitability Test.

2. Sample Preparation and Solid Phase Extraction
   • Prepare samples, IS, Calibrators and QCs, Condition SPE plate,
   • Transfer samples, Calibrators and QCs onto SPE plate,
   • Dry SPE plate under vacuum and N2 flow,
   • Elute SPE plate with DCM (1st extract) and ACN (2nd extract).

3. Performing the HPLC-MS/MS Assay
   • Generate Analyst batch files,
   • Perform HPLC-MS/MS runs with alternating injections,
   • Total runtime: 20 min / sample

4. Data processing
   • Perform data quantification with Analyst software, optionally with MultiQuant,
   • Generate report of analysis.

Results
Method development: Solid Phase Extraction vs. Protein Precipitation
SPE 500 µL:
- Detectable: E2, Aldosterone
- Clean extract: long column life
PP 100 µL:
- Not detectable: E2, Aldosterone
- Dirty extract: short column life

Internal Validation:
1. LOD, LLOQ, ULOQ
2. Linear range, R²>0.99
3. Selectivity
4. Precision (Intra-, Interday)
5. Accuracy (Intra-, Interday)
6. Recovery (70-100% for all compounds)
7. Matrix effects: less than 50% due to clean extract from SPE, fully compensated by isotop labeled IS.
9. Kit stability (shelf life, transport)

External Validation: Proficiency test (Ringversuch DGKL)

External Validation: beta testing in external laboratories
4 different laboratories,
Different HPLC systems: Shimadzu and Agilent,
Samples measured in 5 replicates.
3 levels of concentrations: low, medium, high.
All operators deliver perfectly similar results

Conclusion
With 16 steroid hormones in ist pannel the SteroIDQ® Kit is the most versatile LC-MSMS based platform to deliver high-throughput and accurate quantitative steroid measurement. Extensive internal and external validation have shown that the Kit is very reliable and robust.