# Evaluation of a comprehensive Steroid Panel LC-MS Kit for the measurement of 17 steroids hormones and dexamethasone in a routine clinical laboratory

**Application Note**

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## Abstract

The presented study conducted at the children's hospital in Erlangen (Kinder- und Jugendklinik, Universitätsklinikum Erlangen, Germany) focuses on the evaluation of the clinical performance of the in-house method in comparison to a commercially available CE-IVDD kit solution, the Steroid Panel LC-MS from Tecan.

For clinical performance, the established in-house method was compared to the Tecan kit using serum samples with different clinical backgrounds. 17 steroids and dexamethasone were quantified in clinically relevant serum samples and in ring trial samples from SKML. An excellent correlation ($r > 0.96$) between the commercial method and the in-house method has been observed for eleven different steroids (testosterone, 17-OH-progesterone, progesterone, 11-deoxycortisol, androstenedione, corticosterone, cortisone, cortisol, DHEAS, aldosterone and 11-deoxycorticosterone). The EQAS samples from SKML (testosterone, 17-OH-progesterone, progesterone, cortisol, 11-deoxycortisol, androstenedione, DHEAS, aldosterone, estradiol, DHEA and estrone) were measured to evaluate the clinical bias of the mentioned steroids. In this scope, the correlation between SKML target values and the results gained by Steroid Panel LC-MS was found with $r > 0.94$, demonstrating the reliable quantification of all measured analytes.

The results obtained with the commercial Steroid Panel LC-MS kit were in concordance with the established in-house method. Additionally, this assay allows the clinicians to gain a broader clinical picture of the patients, as it includes 17 steroids and dexamethasone which is helpful for the diagnosis and the understanding of the endocrine disorders.

## Introduction

Liquid Chromatography-Mass Spectrometry (LC-MS) allows for precise identification and quantification of a broad spectrum of analytes. Especially in the steroid analysis, LC-MS became the gold standard over the last years providing valuable insights into hormonal profiles and potential health implications. The benefits of measuring steroids via LC-MS include high sensitivity and specificity enabling accurate identification and quantification of multiple steroids using only one extraction and/or measurement. Multi-steroid analysis offers a comprehensive view of hormonal status, aiding in research, clinical diagnostics, and monitoring conditions such as hormonal imbalances or endocrine disorders. LC-MS also allows for simultaneous detection of various steroid classes with improved precision compared to traditional methods.

LC-MS is recommended for measuring circulating steroids, however, there is nearly no commercial solution available nor an external quality assessment scheme (EQAS) for all clinically relevant steroids.[1]-[3]

This study aims to compare the results of the laboratory-specific procedure performed in the Erlangen Children's Hospital to the commercially available kit from Tecan for the measurement of 17 circulating steroids and dexamethasone. Additionally, an evaluation of the clinical bias of the mentioned kit by using external quality control samples from Stichting Kwaliteitsbewaking Medische Laboratorium diagnostiek (SKML) as a reference was performed. Since no reference measurements and/or ring trial samples are available for all steroids included in the commercial kit, the results of the serum samples were set in a clinical context afterwards.

**Material and Methods**

For method comparison, the in-house method from the Department of Pediatrics and Adolescent Medicine, University Hospital, Erlangen, Germany (in the following: Universitätsklinikum Erlangen), as well as the Steroid Panel LC-MS method described in the Instruction for use (IFU) of the product (Steroid Panel LC-MS, Cat# 30191875, IFU Version 2022-06, CE-IVDD, Tecan, IBL International GmbH) were used.

The in-house method established in the Universitätsklinikum Erlangen has been published already.[1]-[3],[5] The lab implemented its own sample preparation protocol as well as an acquisition and quantitation method incorporating commercial calibrators for their multi-steroid LC-MS/MS assay and regularly releases results for 17-OH-progesterone, androstenedione, cortisol, testosterone, 11-deoxycortisol, 11-deoxycorticosterone, 21-deoxycortisol, corticosterone, cortisone, progesterone, and aldosterone.

The Tecan Steroid Panel LC-MS kit was used according to the IFU. The kit solution provides all necessary standards, reagents, and consumables, apart from methanol and ultra-pure water to perform the assay. Additionally, the calibration standards as well as the controls are prepared in human serum and are shipped lyophilized. For sample preparation, only 250 µL of each serum sample, calibrator, and control was used followed by the addition of 50 µL of internal standard. The sample preparation was performed utilizing a Solid Phase Extraction (SPE) allowing the removal of potential interferences. The overview of the steroids included in the kit with their respective calibration ranges is listed in Table 1.

For both methods, an Agilent 1260 Infinity II UPLC System (Agilent, Santa Clara, CA, US) coupled to an API 6500 QTRAP LC-MS/MS System (SCIEX, Danaher Corporation, Washington D. C., US) was used for analysis. The injection volume was 20 µL for both methods. The acquired data were evaluated using MultiQuant Software (SCIEX, Danaher Corporation, Washington D. C., US). The Regression type was chosen as linear with weighting type 1/x. The statistical analysis, namely the Passing-Bablok fit, was performed using Analyse-it (Version 6.15, Analyse-it Software, Ltd.; Leeds, UK).

For method comparison, 13–19 human clinical samples collected for routine measurement at the endocrinological laboratory of the Erlangen Children's Ambulance Hospital were used. These samples are considered to be from affected children suffering from endocrine disorders. Some of the patients underwent ACTH stimulation as part of their clinical diagnosis. All the samples were anonymous and measured in single determination in one batch.

For evaluation of clinical bias, SKML ring trial samples (proficiency samples containing defined concentrations of steroid hormones in serum, panel "Hormones in Serum", including testosterone, 17-OH-progesterone, progesterone, cortisol, 11-deoxycortisol, androstenedione, DHEAS, aldosterone, estradiol, DHEA, estrone and 21-deoxycortisol) were collected from

2022 and measured in single determination in one batch. The overview of the analytes measured in the current study is listed in Table 1.

Table 1: Overview of the analytes with their corresponding calibration ranges with the commercial kit provided by Tecan compared to the in-house method of Universitätsklinikum Erlangen, and information of the expected ranges from the measured SKML ring trial samples. N/A: Non Applicable.

| **Analyte** | **Calibration ranges Steroid Panel LC-MS [ng/mL] (nmol/L)** | **Calibration ranges in-house method by Erlangen [ng/mL] (nmol/L)** | **Ring trial samples from SKML with expected ranges [ng/mL] (nmol/L)** |
|---|---|---|---|
| Testosterone | 0.04-13.4 (0.138-46.5) | 0.053-12.00 (0.183-41.6) | 0.196-11.18 (0.68-38.8) |
| 17-OH-Progesterone | 0.1-12.8 (0.303-38.7) | 0.087-14.5 (0.26-43.9) | 0.23-6.01 (0.70-18.2) |
| Progesterone | 0.1-12.8 (0.318-40.7) | 0.134-14.5 (0.426-46.1) | 0.11-12.73 (0.35-40.5) |
| Cortisol | 2-256 (5.52-706) | 10-279 (27.6-770) | 85.18-349.08 (235-963) |
| 11-Deoxycortisol | 0.1-12.8 (0.20-36.9) | 0.091-13.7 (0.26-39.5) | 0.20-88.76 (0.60-256) |
| Androstenedione | 0.1-12.2 (0.34-42.5) | 0.184-13.50 (0.64-47.1) | 0.40-4.58 (1.4-16.0) |
| DHEAS | 50-6,412 (135-17,376) | 109-5,664 (295-15,349) | 585-6.548 (1,590-17,700) |
| Cortisone | 0.5-64.1 (1.38-177) | 1.03-41.0 (2.85- 113) | N/A |
| Corticosterone | 0.3-38.5 (0.86-111) | 0.531-45.9 (1.53-132) | N/A |
| 11-Deoxycorticosterone | 0.04-5.13 (0.12-15.5) | 0.044-2.91 (0.13-8.81) | N/A |
| 21-Deoxycortisol | 0.1-12.8 (0.289-36.9) | 0.069-5.01 (0.199-14.4) | N/A |
| Aldosterone | 0.1-4.59 (0.27-12.7) | 0.023-2.74 (0.063-6.84) | 0.064-0.39 (0.18-1.09) |
| Estradiol | 0.03-3.85 (0.11-14.1) | N/A | 0.22-0.43 (0.82-1.60) |
| Estrone | 0.01-1.28 (0.037-4.73) | N/A | 0.021-0.048 (0.08-0.18) |
| DHT | 0.15-1.57 (0.51-5.40) | N/A | N/A |
| Dexamethasone | 0.50-64.1 (1.379-176) | N/A | N/A |
| 17-OH-Pregnenolone | 0.3-38.47 (0.909-116) | N/A | N/A |
| DHEA | 1-45.9 (3.47-159) | N/A | 0.92-6.83 (3.2-23.7) |

## Results and discussion:

Method comparison based on clinical samples

For both methods, clinical samples were measured for the determination of the concentration of testosterone, 17-OH-progesterone, progesterone, 11-deoxycortisol, 21-deoxycortisol, androstenedione, corticosterone, cortisone, cortisol, and 11-deoxycorticosterone. Additionally, up to 12 ring trial samples have been included in order to cover DHEA-s and aldosterone as well. After quantitative analysis, the Passing-Bablok fits were plotted to evaluate the comparison of the used methods (see Figure 2). The Passing-Bablok fit takes into

consideration at least nine samples for each analyte. Values under LoQ were excluded in the method comparison. As only one sample was measured above the LoQ for 21-deoxycortisol for the in-house method, this parameter is not represented graphically. The presented results show a good correlation for all the other measured analytes: The correlation *r* for each analyte is given in Table 2 and exceeds 0.96 for all the 11 steroids measured. Overall, it can be stated that the in-house method and the Steroid Panel LC-MS achieved comparable results for the mentioned analytes, leading to the same clinical statement. A deeper look at the clinical interpretation is made in the corresponding chapter.

Table 2: Method Comparison – Correlation of in-house method and commercial kit quantified by r. Data generated via Analyse-It. *Data solely based on SKML samples.

| **Analyte** | ***n*** | ***r*** |
|---|---|---|
| Testosterone | 31 | 0.982 |
| 17-OH-Progesterone | 29 | 0.994 |
| Progesterone | 27 | 0.996 |
| 11-Deoxycortisol | 29 | 0.962 |
| Androstenedione | 28 | 0.999 |
| DHEAS* | 10 | 0.998 |
| Aldosterone* | 9 | 0.971 |
| Cortisol | 31 | 0.990 |
| 11-Deoxycorticosterone | 13 | 0.999 |
| Cortisone | 19 | 0.997 |
| Corticosterone | 24 | 0.995 |

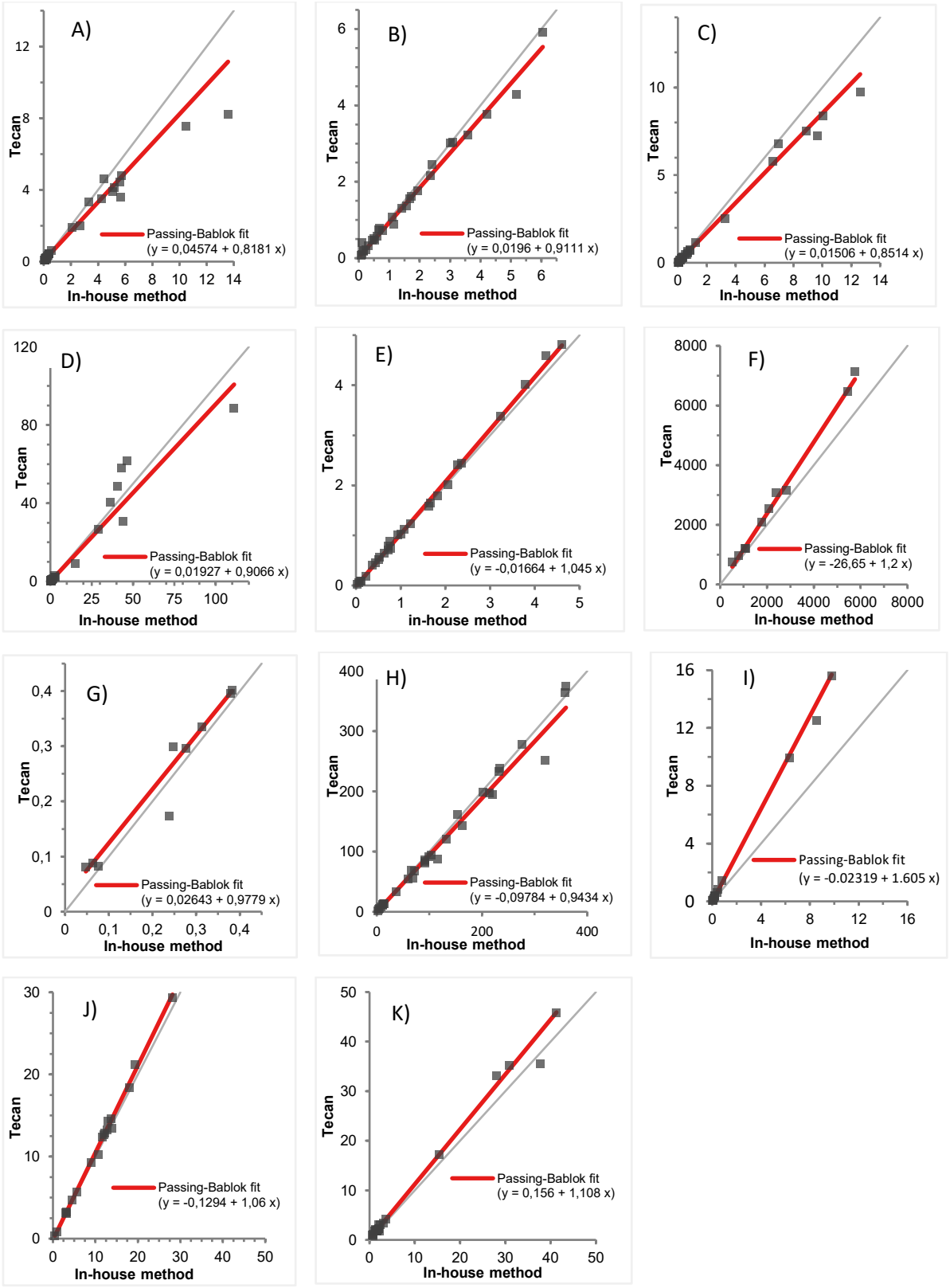


Figure 2: Method comparison of the in-house method to Steroid Panel LC-MS by Tecan. Passing-Bablok fit based on at least 9 samples is shown for each analyte separately; all values are in ng/mL: (A) Testosterone, (B) 17-Hydroxyprogesterone, (C) Progesterone, (D) 11-Deoxycortisol, (E) Androstenedione, (F) DHEAS, (G) Aldosterone, (H) Cortisol, (I) 11-Deoxycorticosterone, (J) Cortisone and (K) Corticosterone.

Bias evaluation based on SKML ring trial samples

In order to evaluate the clinical bias, ring trial samples from SKML were analyzed with Steroid Panel LC-MS at Universitätsklinikum Erlangen. Figure 3 shows the Passing-Bablok fit for testosterone, 17-OH-progesterone, progesterone, cortisol, 11-deoxycortisol, androstenedione, DHEAS, aldosterone, estradiol, DHEA and estrone comparing the concentration gained with Tecan kit to the target value given from SKML. All the results have shown a good correlation between measured and target values, with *r* greater than 0.94 (see Table 3).

Table 3: Evaluation of clinical bias based on the measurement of SKML samples.

| **Analyte** | ***n*** | **SKML ranges** | | **Correlation *r*** |
|---|---|---|---|---|
| | | **Gained with Tecan kit [nmol/L]** | **Target values [nmol/L]** | |
| Testosterone | 12 | 0.76-28.38 | 0.68-38.8 | 0.997 |
| 17-OH-Progesterone | 12 | 0.67-17.89 | 0.70-18.20 | 0.993 |
| Progesterone | 12 | 0.14-40.12 | 0.35-40.50 | 0.966 |
| Cortisol | 12 | 223-1033 | 235-963 | 0.982 |
| 11-Deoxycortisol | 12 | 0.60-318 | 0.60-256.17 | 1 |
| Androstenedione | 12 | 1.43-16.77 | 1.4-16.0 | 1 |
| DHEAS | 10 | 2,021-19,341 | 1,590-17,700 | 0.991 |
| Aldosterone | 10 | 0.22-1.112 | 0.18-1.09 | 0.981 |
| Estradiol | 11 | 0.06-1.49 | 0.82-1.60 | 0.992 |
| Estrone | 11 | 0.09-0.18 | 0.08-0.18 | 0.948 |
| DHEA | 12 | 2.43-23.95 | 3.2-23.7 | 0.980 |

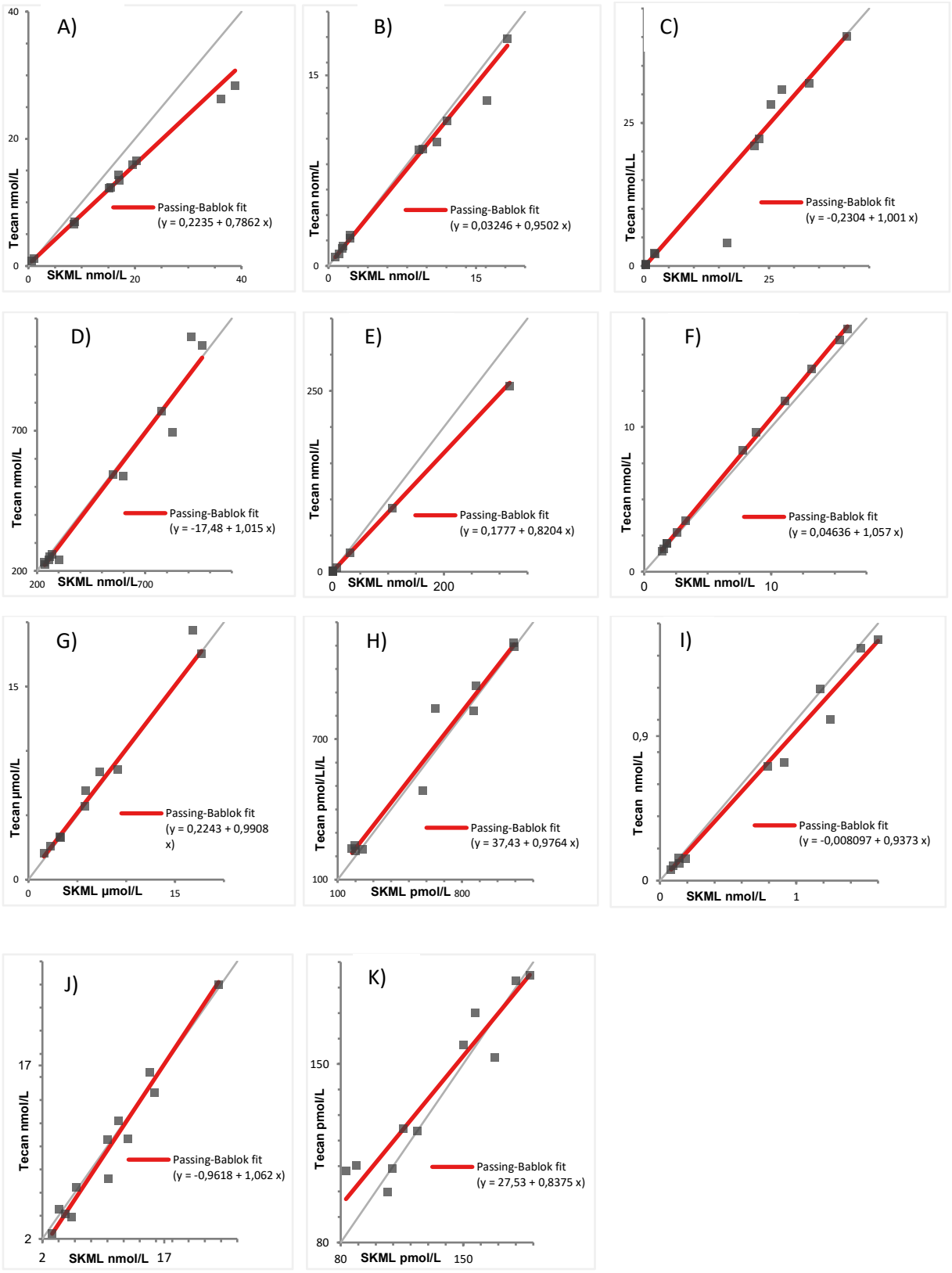


Figure 3: Passing-Bablok plot for the evaluation of bias of Steroid Panel LC-MS using SKML ring trial samples (at least 9 samples for each analyte), different plots refer to different steroids: (A) Testosterone, (B) 17-Hydroxyprogesterone, (C) Progesterone, (D) Cortisol, (E) 11-Deoxycortisol, (F) Androstenedione, (G) DHEAS, (H) Aldosterone, (I) Estradiol, (J) DHEA and (K) Estrone.

Analysis of clinically relevant samples

The Tecan steroid kit allows the determination of 17 natural and circulating steroids as well as dexamethasone. In the following chapter, the results of the quantitative analysis obtained with the Steroid Panel LC-MS for clinically relevant serum samples were set in a clinical context. As these samples were collected in a clinical endocrine laboratory, they are considered to be from an affected population (not apparently healthy). Especially, five samples have shown particular patterns and values for specific steroids falling outside the expected normal ranges.

For 11-deoxycorticosterone, the samples have shown values up to 27.86 ng/mL using the Tecan kit, which is a 100-fold increase from the normal range of 0.01-0.27 ng/mL[6]. The values gained with the in-house method also showed elevated values for 11-deoxycorticosterone. The values obtained for 11-deoxycortisol displayed a similar pattern for both methods being very high, while corticosterone and cortisol values were very low for both methods.

Such a pattern is expected to be observed after Metyrapone treatment. The latest, trade name Metopirone®, is a drug used in the diagnosis of adrenal insufficiency and occasionally in the treatment of Cushing's syndrome (hypercortisolism). Metyrapone blocks cortisol synthesis in healthy humans by inhibiting steroid 11 ß-hydroxylase in the cortex of suprarenal gland. This stimulates adrenocorticotropic hormone (ACTH) secretion by negative feedback, which results in an increase of 11-deoxycortisol and 11-deoxycorticosterone levels.[5] The figure 4 describes the steroid pathway and shows the interdependency of the steroids and enzymes involved in their conversions.

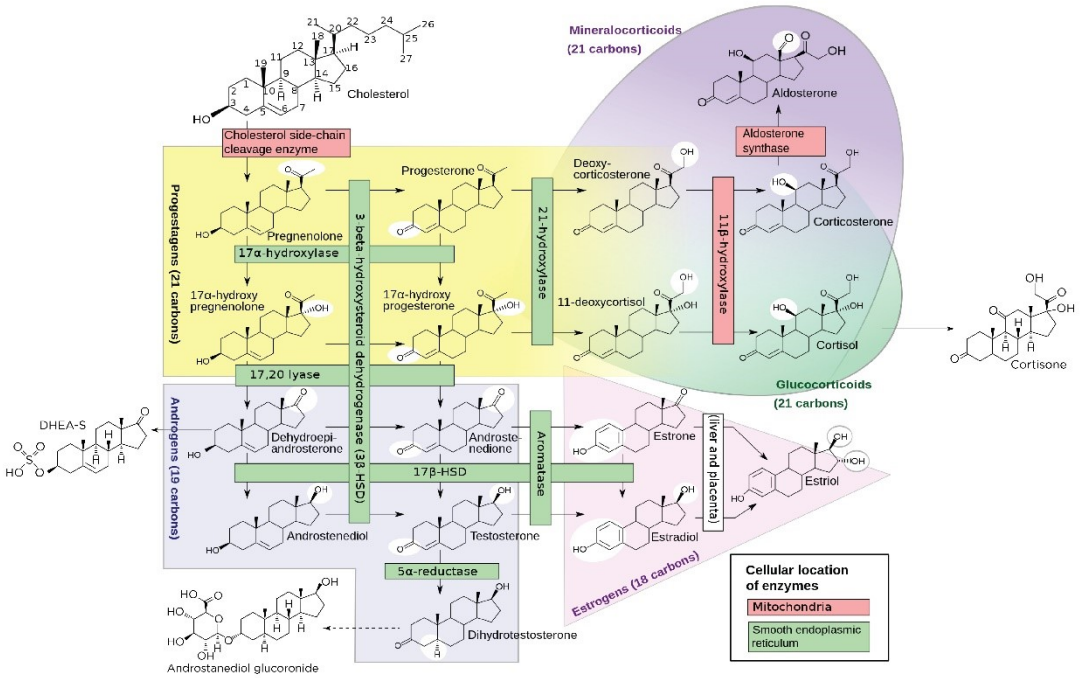


Figure 4: Overview of the steroid pathway, reworked from the "Diagram of the pathways of human steroidogenesis", WikiJournal of Medicine 1 (1). DOI:10.15347/wjm/2014.005. ISSN 20018762.

For 21-deoxycortisol, most of the samples were under the limit of quantitation of the Tecan kit as well as with the in-house method. Accordingly, the clinical interpretation of the values obtained with the Tecan kit is the same as the in-house method used in routine diagnostics.

The exemplary discussion for the mentioned steroids shows that the results obtained with both commercial and in-house methods lead to the same clinical interpretation. Table 4 lists the

values obtained for all steroids included in the Tecan kit (split in Table 4-1 and 4-2 for better overview).

Table 4-1: Concentration of steroids in five different serum samples after Metyrapone treatment. The grey underlined values were acquired using the Tecan kit whereas the other values were measured with the in-house method from Universitätsklinikum Erlangen. Bold values were elevated for the analyzed patient population.

| No. | Method | Concentration [ng/mL] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | P4 | 17-OHP4 | DOC | 11-DF | 21-DF | B | F | ASD | E |
| 1 | In-House Erlangen | 0.824 | 2.36 | **15.31** | **46.23** | < LoQ | < LoQ | 11.03 | 2.06 | 5.55 |
| | Steroid Panel LC-MS | 0.766 | 2.16 | **27.86** | **61.61** | < LoQ | 0.293 | 10.31 | 2.00 | 5.69 |
| 2 | In-House Erlangen | 0.316 | 1.15 | **6.32** | **29.09** | < LoQ | < LoQ | 5.82 | 0.726 | 3.20 |
| | Steroid Panel LC-MS | 0.295 | 0.87 | **9.91** | **26.77** | < LoQ | 0.315 | 5.72 | 0.708 | 3.02 |
| 3 | In-House Erlangen | 0.485 | 1.09 | **11.26** | **36.35** | < LoQ | < LoQ | 12.84 | 1.02 | 9.01 |
| | Steroid Panel LC-MS | 0.479 | 1.06 | **21.30** | **40.35** | < LoQ | 0.707 | 12.91 | 1.02 | 9.22 |
| 4 | In-House Erlangen | 0.294 | 1.41 | **8.55** | **40.61** | < LoQ | < LoQ | 3.92 | 1.83 | 2.94 |
| | Steroid Panel LC-MS | 0.299 | 1.30 | **12.50** | **48.60** | < LoQ | 0.383 | 4.32 | 1.80 | 3.10 |
| 5 | In-House Erlangen | 0.342 | 1.56 | **9.79** | **42.73** | < LoQ | < LoQ | 11.04 | 1.23 | 4.58 |
| | Steroid Panel LC-MS | 0.310 | 1.36 | **15.57** | **57.99** | < LoQ | 0.401 | 11.43 | 1.24 | 4.70 |

Abbreviations: P4 – progesterone, 17-OHP4 – 17-OH-progesterone, DOC – 11-deoxycorticosterone, 11DF – 11-deoxycortisol, 21DF – 21-deoxycortisol, B – corticosterone, F – Cortisol, ASD – androstenedione, E – cortisone, LoQ – Limit of Quantitation.

Table 4-2: Concentration of steroids in five different serum samples after Metyrapone treatment. The grey underlined values were acquired using the Tecan kit whereas the other values were measured with the in-house method from Universitätsklinikum Erlangen. Bold values were elevated for the analyzed patient population. Values for the in-house method of Universitätsklinikum Erlangen marked with N/A are not available, as no in-house method was implemented for routine laboratory diagnostics.

| No. | Method | Concentration [ng/mL] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | DHT | DHEAS | DHEA | T | A | E1 | E2 | 17-OHP5 | DST |
| 1 | In-House Erlangen | N/A | N/A | N/A | 0.293 | N/A | N/A | N/A | N/A | N/A |
| | Steroid Panel LC-MS | < LoQ | 869 | 4.05 | 0.323 | < LoQ | 0.031 | < LoQ | 7.60 | < LoQ |
| 2 | In-House Erlangen | N/A | N/A | N/A | 0.111 | N/A | N/A | N/A | N/A | N/A |
| | Steroid Panel LC-MS | < LoQ | 273 | < LoQ | 0.152 | < LoQ | 0.042 | 0.057 | 0.477 | < LoQ |
| 3 | In-House Erlangen | N/A | N/A | N/A | 0.165 | N/A | N/A | N/A | N/A | N/A |
| | Steroid Panel LC-MS | < LoQ | 6231 | 6.77 | 0.181 | < LoQ | 0.034 | 0.014 | 12.2 | < LoQ |
| 4 | In-House Erlangen | N/A | N/A | N/A | 0.386 | N/A | N/A | N/A | N/A | N/A |
| | Steroid Panel LC-MS | < LoQ | 3048 | 3.95 | 0.414 | < LoQ | 0.056 | 0.029 | 3.64 | < LoQ |
| 5 | In-House Erlangen | N/A | N/A | N/A | 0.166 | N/A | N/A | N/A | N/A | N/A |
| | Steroid Panel LC-MS | < LoQ | 769 | < LoQ | 0.148 | 0.045 | 0.034 | < LoQ | 1.60 | < LoQ |

Abbreviations: DHT – dihydrotestosterone, DHEAS – dehydroepiandrosterone-sulfate, DHEA – dehydroepiandrosterone, T – testosterone, A – aldosterone, E1 – Estrone, E2 – Estradiol, 17-OHP5 – 17-OH-pregnenolone, DST – dexamethasone, N/A – not available, LoQ – Limit of Quantitation.

However, it has to be pointed out, that not all the steroids included in the Tecan kit are routinely measured at the Universitätsklinikum Erlangen, nor external quality assessment scheme is available for all parameters. Accordingly, the clinical performance for the missing steroids 17-OH-Pregnenolone, DHT and Dexamethasone is separately evaluated as follows:

The 17-OH-Pregnenolone concentrations have been measured for all clinical samples between 0.3 and 16.90 ng/mL. These values are higher than the expected range between (0.41 to 4.80 ng/mL) for the children in healthy populations. Particularly, the 17-OH-Pregnenolone values for the five highlighted samples in table 4-2 were elevated and above the normal ranges, showing concentrations up to 12.2 ng/mL. Such high concentrations can be expected as consequence of the Metyrapone test, as 17-OH-Pregnenolone is a precursor of 17-OH-Progesterone and 11-deoxycortisol ultimately (Figure 4).

The concentration of dihydrotestosterone (DHT) in the analyzed serum samples is in a range of 0.13–0.38 ng/mL. These values met the normal range for individuals under 20 years (< 0.93 ng/mL).[8] DHT values in children are not expected to be elevated,[8] accordingly, the results obtained with the Tecan kit are coherent. Including this parameter in steroid profiles represents a new opportunity for the clinical laboratory to enhance the clinical picture of the patient.

For dexamethasone (DST), all samples measured with the Tecan kit show values below LoQ, which was expected as the patients coming to the clinic are not expected to be under treatment. Nevertheless, the steroid kit is able to measure the affected population since the calibration range is 0.50–64.1 ng/mL and dexamethasone concentration of patients subjected to 1 mg DST is expected to reach up to 20.2 ng/mL (median 4.8 ng/mL).[4] The clinical performance of dexamethasone in the Steroid Panel LC-MS is confirmed by F. Ponzetto *et al.* showing the ability to measure affected populations.[7]

Given the strong association of steroid hormones and endocrine disorders like adrenal insufficiency, cushing's syndrome, and congenital adrenal hyperplasia e.g., steroid testing has become an integral part of diagnostic procedures and is recommended by many clinical practice guidelines.[5] The advantage of the current Tecan Steroid Panel LC-MS is the comprehensive analysis of 17 circulating steroids and dexamethasone. This allows the measurement of a complete steroid profile for the patient and as such is an aid to diagnosis and monitoring patients with suspected or confirmed endocrine disorders.

## Conclusion and Outlook

This study performed a method comparison study between the implemented in-house method for clinical routine measurements of Universitätsklinikum Erlangen and the Steroid Panel LC-MS by Tecan. A good correlation was found between both methods based on clinical samples. To verify these results, in the future, a method comparison study based on CLSI guidelines including at least 40 clinical samples should be performed. Even without this larger number of samples, both methods allow the reliable quantification of the aforementioned steroids due to the shown clinical performance. Clinical samples can be analyzed reliably and therefore both methods meet the laboratory needs. Furthermore, this can be confirmed by the performed accuracy experiments: The presented study showed an excellent agreement between measurements of 11 different steroids with SKML ring trial samples.

The results obtained with the commercial Steroid Panel LC-MS kit were in concordance with the established in-house method. Additionally, this assay allows the clinicians to gain a broader clinical picture of the patients, as it includes 17 steroids and dexamethasone which is helpful for the diagnosis and the understanding of the endocrine disorders. Particularly the addition of new parameters like DHT, Dexamethasone and 17-OH-Pregnenolone are beneficial for the investigation of the patient's hormone status.

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