Steroids in Translational Research – From Mouse to Man with Metabolic Phenotyping

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Metabolic phenotyping enables important insights into pathways and biological processes. By quantification of endogenous metabolites in biological matrices, essential information about disease phenotypes and biological surrogate markers can be obtained. This metabolic information is not restricted to a specific species and is thus highly applicable in translational research studies, enabling transition of major aspects of preclinical and clinical knowledge from bench to bedside.

Steroid hormones are important regulators of physiological functions, e.g. sexual dimorphism, modulation of glucose metabolism, regulation of water and mineral function in the body. U(H)PLC-MS/MS techniques can be used to quantify multiple steroids simultaneously with high specificity and low inter-laboratory variability. Biocrates developed an U(H)PLC-MS/MS research Kit that offers the opportunity to quantify 17 steroid hormones with high throughput and excellent sensitivity.

The Absolute*IDQ*® Stero17 Kit allows the standardized and quality controlled quantitation of steroids in biological matrices.

	Waters Xevo TQ-S		ABSciex 5500 QTRAP								
Steroids	**human serum female 500 µL**	**human serum female 250 µL**	**human serum female 200 µl**	**human serum male 200 µl**	**human plasma female 250 µl**	**human plasma male 250 µl**	**mouse EDTA plasma 250 µL**	**mouse EDTA plasma 100 µL**	**mouse EDTA plasma 50 µL**	**amniotic fluid mouse 150 µl**	**bovine serum female 250 µl**
11-Deoxycorticosterone (11-DOCSt)	++	++	+/-	+/-	+/-	+/-	++	++	++	++	+
11-Deoxycortisol (11-DOC)	++	++	++	++	++	++	-	-	-	+	+
17α–Hydroxyprogestrone (17-OHP)	++	++	++	++	++	++	-	-	-	-	+
Aldosterone	+/-	+/-	++	++	+/-	+/-	++	++	++	++	+/-
Androstenedione	++	++	++	++	++	++	+/-	-/+	-	-/+	+/-
Androsterone	+/-	++	+	++	++	++	+/-	-/+	-	+/-	++
Corticosterone	++	++	++	++	++	++	++	++	++	++	+/-
Cortisol	++	++	++	++	++	++	-	-	-	-	++
Cortisone	++	++	++	++	++	++	-	-	-	-	++
DHEA	++	++	++	++	++	++	-	-	-	-	++
DHEAS	++	++	++	++	++	++	-	-	-	-	-
Dihydrotestosterone (DHT)	++	++	++	++	+	++	-	-	-	-	-/+
Estrone (E1)	+	++	++	++	-/+	-/+	-	-	-	++	++
Estradiol (E2)	+	+	+	+	-/+	+/-	-	-	-	-/+	+/-
Etiocholanolone	-/+	+/-	+/-	+/-	-/+	+/-	-	-	-	-	++
Progesterone	++	++	++	+/-	++	+/-	++	++	++	+	++
Testosterone	++	++	++	++	++	++	++	++	++	-/+	+/-

-	Not detected or near LOD
-/+	Detected but concentration mostly < LLOQ
+/-	Concentration around LLOQ
+	Concentration slightly above LLOQ
++	Concentration well above LLOQ

Figure 1: Steroid profile in human serum and plasma, mouse plasma and amniotic fluid, and bovine serum

To investigate species-dependent patterns of steroid hormones, we determined the steroid concentration in human, mouse and bovine serum and/or EDTA plasma (figure 1). While cortisol, cortisone and DHEA were found in human and bovine matrix, these analytes were

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not detected in mouse samples. The analysis of rat serum revealed similar findings (data not shown), supporting the data obtained from mouse experiment. High concentrations of etiocholanolone were observed in bovine serum.

A challenging factor in steroid analysis, especially in small animal studies, is the sample volume. The higher concentrated steroids 11-Deoxycorticosterone (11-DOCSt), aldosterone, corticosterone, progesterone and testosterone were detected in mouse plasma sample volumes of 50 – 250 µL. However, androstenedione and androsterone could not be detected using a sample volume of 50 µL. Increase of the sample volume to 250 µL enabled quantitation of these steroids with values around LLOQ.

A similar steroid profile was observed in female and male humans in serum (sample volume 200 µL). Differences were observed for the sex specific hormones estrone, progesterone, DHT and testosterone. These differences are partially not apparent in figure 1 as most steroids are well above LLOQ. The sex dependent characteristics of steroid profiles in humans were observed in female and male plasma with sample volumes of at least 250 µL.

Comparison of different studies of steroid hormones revealed that the steroid profile strongly depends on the analyzed sample cohort. Differences were observed in levels of 11-DOCSt, aldosterone, androsterone, DHT, E2, E1 and etiocholanolone. These findings are independent of sex, matrix and sample volume.

In conclusion, the Absolute*IDQ*® Stero17 Kit enables assessment of steroid hormone profile in various species, providing essential information for translational research.

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