

## Establishment of reference values for 6 steroids in serum by LC-MS/MS

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### Background:

Steroid hormones play a crucial role in controlling metabolism, immune functions and inflammation. Modifications in steroid profiling reflect disease status and help research into a wide number of health disorders, including congenital adrenal hyperplasia, Cushing's disease and polycystic ovarian disease. However, the quantification of steroids by immunoassays may underestimate some of them. To overcome these limitations, we obtained the new reference range values with an LC-MS/MS method based on the MassChrom® kit. In the present work a reference interval for serum androstenedione, corticosterone, cortisol, aldosterone and 11-desoxycortisol for a healthy Belgian population is presented.

### Materials and Methods:

✓ For the serum reference interval study, recruited participants were normotensive (clinical blood pressure <140/90 mmHg), without antihypertensive or corticosteroid treatment, non-smokers and did not take any oral contraception. All participants gave informed consent and were fasting.

✓ We enrolled 55 healthy Caucasian volunteers (36 F: mean age 43.6 ± 11.7 yo and 20 M: mean age 38 ± 13 yo)

✓ Blood samples were centrifuged immediately after the draw (morning) for 10 minutes at 2500 g, aliquoted and stored frozen at -80° C before further analysis. Study was approved by ethic committee of the university in Liege.

✓ The analysis was performed with the MassChrom® kit from Chromsystems (Heimbürgstrasse, Munich, Germany) using:

- panel 1 for corticosterone, cortisol, aldosterone and 11-desoxycortisol
- panel 2 for androstenedione

on a QTrap 6500 (Sciex, Framingham, Massachusetts, USA) (Fig.1).

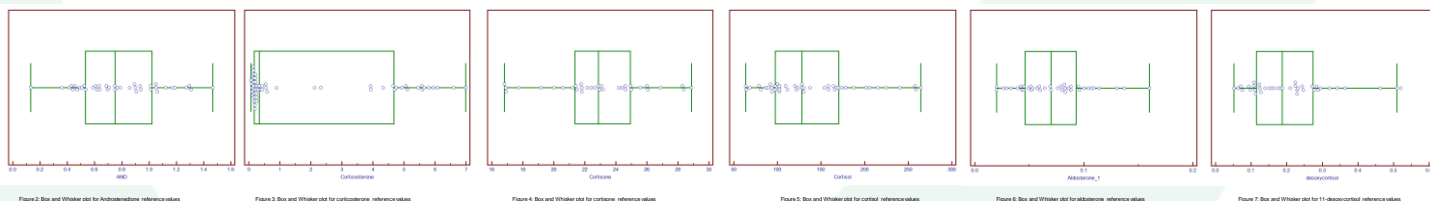
✓ Statistical analysis was performed using Medcalc (Mariakerke, Belgium) software.

### Results:

No difference for the gender was observed for these 6 steroids. The reference values were obtained with a robust and validated method, being:

- 0.18-1.37 µg/L for androstenedione (Fig.2)
- <6.86 µg/L for corticosterone (Fig.3)
- 16.5-28.82 µg/L for cortisol (Fig.4)
- 30.48-230.98 µg/L for cortisol (Fig.5)
- <156 ng/L for aldosterone (Fig.6)
- <0.44 µg/L for 11-desoxycortisol (Fig.7)

Figure 1: Qtrap 6500



### Conclusions:

We have established new reference intervals in LC-MS/MS for 6 steroids in serum. A low number of volunteers limit the validity of our study results. However, we will continue the recruiting to obtain a larger number of reference values to be in line with CLSI recommendations.