

TurboFlow-LC-MS/MS method for quantification of five androgens in children and comparison with immunoassays

Tue Søeborg, Hanne Frederiksen, Trine Holm Johannsen, Anders Juul, Anna-Maria Andersson

Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Denmark

Introduction

Diagnosis and management of children with sex steroid disorders requires fast and simultaneous assessment of sex steroids in serum at low concentrations and on small sample volumes. Therefore, we developed a sensitive and selective TurboFlow-LC-MS/MS method for quantification of dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEAS), 17 α -hydroxyprogesterone (17-OHP), Δ 4-androstenedione (Adione) and testosterone (T) (Fig. 1) in serum from pre-pubertal children [1].

Study samples

Thirty-eight serum samples were used for the method validation and 186 serum samples were used for comparison between the current LC-MS/MS method and immunoassays. The samples were from healthy Danish children participating in the COPENHAGEN Puberty Study [2;3]. Briefly, the children were physically examined for signs of puberty including measurement of testis volume, palpation of breast tissue and presence of pubertal hair. The samples included for validation of the current method were from children showing no signs of puberty.

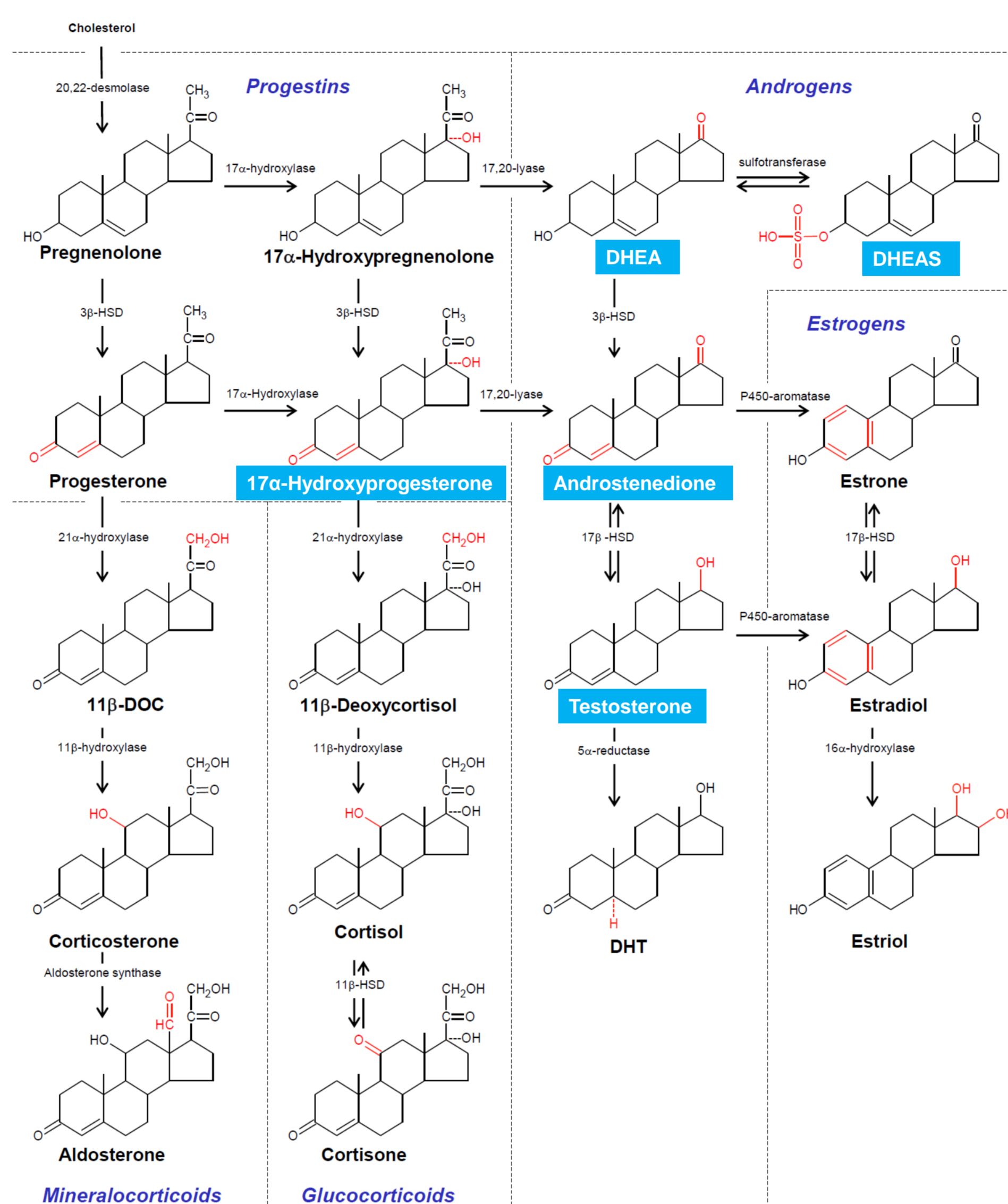


Fig. 1: Steroidogenesis including the five steroids included in the current study.

Compound	Linear range (nM)	R ²	Intra-day RSD (%)	Inter-day low RSD (%)	Inter-day high RSD (%)	LOQ (nM)
DHEA	0.24 – 121	0.9997	13.8	15.7	8.6	0.88
DHEAS	33 – 17097	0.9995	4.6	7.1	8.3	48
17-OHP	0.062 – 32	0.9987	7.4	10.9	9.9	0.19
Adione	0.14 – 73	0.9996	5.2	11.0	11.1	0.18
T	0.071 – 36	0.9999	5.9	10.0	5.7	0.10

Table 1: Validation data.

Sample preparation

Serum was mixed with ZnSO₄ in MeOH containing deuterated internal standards. After centrifugation for 10 min (see Fig. 2) an aliquot of the supernatant was injected into the LC-MS/MS system as previously described [1].

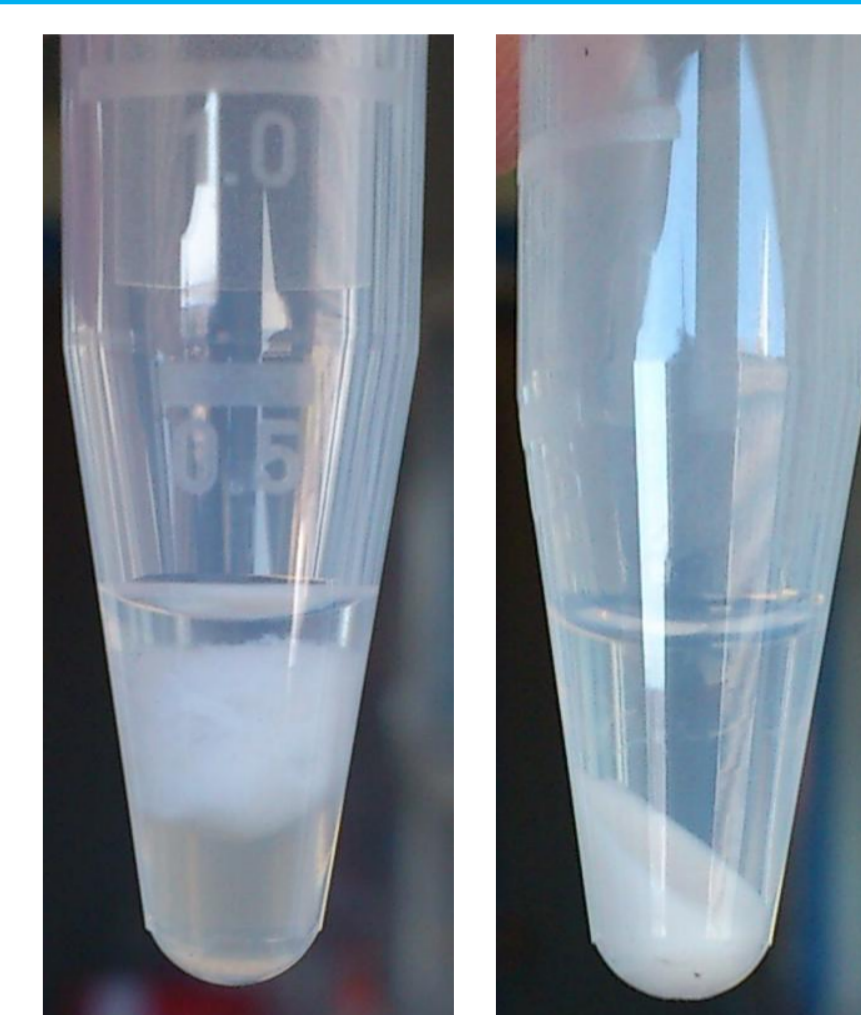


Fig. 2: Serum and ZnSO₄ before (left) and after (right) mixing and centrifugation.

Validation

Validation data is given in Table 1. All data presented in Table 1 (except inter-day relative standard deviations (RSDs)) was produced using virtually steroid-free serum spiked with standards and internal standards. Limits of quantitation (LOQs) were determined according to the International Conference on Harmonisation (ICH) guideline [4]. Inter-day RSDs were based on repeated analysis of serum pools from men and women, respectively. The virtually steroid-free serum was also used for a matrix effect study comparing commercially available stripped serum and milli-Q water (for details, see [1]).

Comparison with immunoassays

Serum samples from healthy Danish children (8 – 15 years old) were used for comparison of results obtained using the current LC-MS/MS method and immunoassays for DHEAS and Adione (both Immulite 2000) and T (Siemens Coat-A-Count) (for details, see [5]). The results are given in Fig. 3A-C.

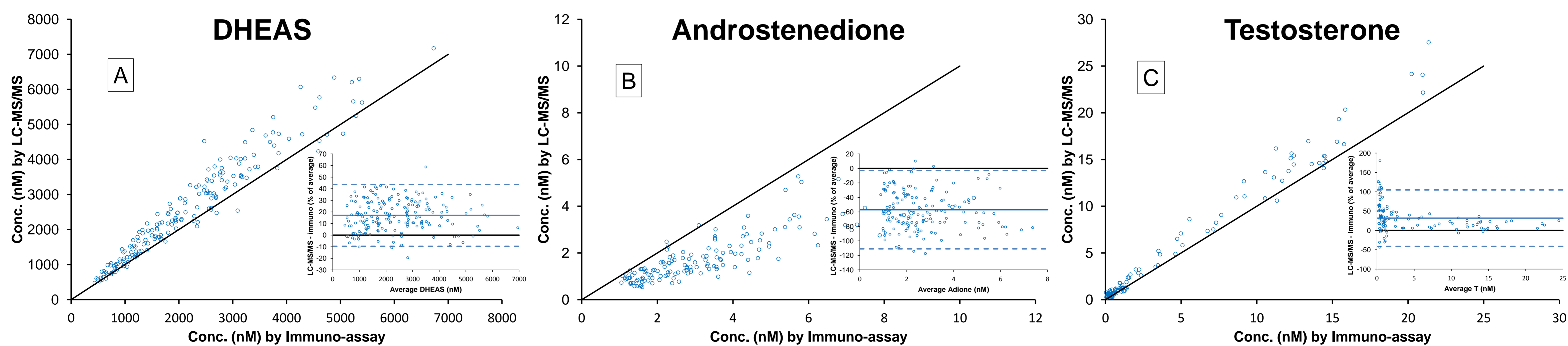


Fig. 3A, B and C: Correlation between immunoassays and the current LC-MS/MS method. Bland-Altman plots are inserted with mean (solid line) and \pm 2SD (dashed lines). Identity lines at $y = 0$ are also included. 186 serum samples were analysed.

References

- Søeborg et al. Clin Chim Acta 2013; 419:95-101.
- Aksglæde et al. Pediatrics 2009; 123:e932-e939
- Sørensen et al. J Clin Endocrinol Metab 2010; 95:263-70
- ICH, 2005. Validation of analytical procedures. Text and methodology (<http://ich.org/>)
- Mouritsen et al. Eur J Endocrinol 2013; 168:129-36
- Fanelli et al. Steroids 2011; 76:244-53

Discussion and conclusion

Immunoassays tend to overestimate the serum concentration of Adione when compared to LC-MS/MS based assays [6], which was also the case in this study (see Fig. 4B). The estimated concentrations of DHEAS and T were more comparable between the two techniques (Fig. 4A and 4C) but with large differences between the two techniques at low concentrations for T. DHEAS could be quantified in all samples with both techniques, while Adione and T could be quantified in 78 % and 61 % of the samples, respectively using immunoassays and in 98 % and 94 % of the samples, respectively using the current LC-MS/MS method. The presented method is suitable in a clinical setting for simultaneous quantification of five steroids important for management of children with disorders of sex development and steroid biosynthesis defects and a necessity for quantification of - at least - Adione and T in children as an alternative to conventional immunoassays.