One Small Draw, the Whole Endocrine Map: Multiplexed Steroid, Pituitary, and Vitamin D Profiling from a Single Capillary Collection

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CLIA ID 19D2139173 · correspondence — info@clinicorelabs.com · specimen: one small-volume (~600 µL) capillary red-top collection

ABSTRACT

Rationale. The endocrine system is a connected network of biosynthetic pathways and feedback loops, yet it is usually tested one hormone at a time, on venous blood. Two developments change what a single specimen can deliver: liquid chromatography–tandem mass spectrometry (LC–MS/MS), which measures large panels of structurally similar analytes together and specifically, and microsampling, which brings that panel within reach of a small capillary volume.

What the panel covers. From a single ~600 µL capillary collection, Clinicore maps adrenal steroidogenesis (mineralocorticoids, glucocorticoids, and their precursors), the sex-steroid pathway with its binding protein, the gonadotropins and selected pituitary hormones, a metabolic marker, and vitamin D — dozens of analytes spanning the endocrine network.

Why it is powerful. Because precursors and products are measured together, product-to-precursor ratios read the enzymes of steroidogenesis, and cross-analyte ratios — cortisol:cortisone, DHT:testosterone, estradiol:estrone, free-testosterone/SHBG, LH:FSH, testosterone:cortisol, and others — add interpretation no single value provides. Small volume enables access and serial monitoring; LC–MS/MS provides specificity immunoassays lack at the low concentrations that matter.

Conclusion. A single small-volume collection yields an integrated endocrine map — and, just as importantly, the ratios that read it.

Keywords: LC–MS/MS · steroid profiling · microsampling · capillary collection · steroidogenesis · product-to-precursor ratios · free testosterone / SHBG · LH:FSH · cortisol:cortisone · 25-hydroxyvitamin D

1 Background

Hormones do not act in isolation. Adrenal and gonadal steroids share a common biosynthetic tree; binding proteins set what fraction is bioavailable; pituitary signals drive the peripheral glands; and metabolic and vitamin-D status modulate the whole. Testing that samples one hormone at a time, on separate venous draws, sees the network only in fragments. This review describes an alternative made practical by two technologies — multiplexed LC–MS/MS measurement and small-volume microsampling — in which a single capillary collection maps the endocrine network broadly enough to read not just individual hormones but the relationships among them.

2 One collection, the whole map

Multiplexed LC–MS/MS assays routinely quantify large steroid panels from a single specimen: published methods measure fifteen gluco- and mineralocorticoid-pathway steroids in one run, and thirteen steroids simultaneously in clinical cohorts.[2,3] The same chemistry works from very small volumes — a validated dried-blood-spot method quantifies nine steroids (cortisol, 17-hydroxyprogesterone, 11-deoxycortisol, 21-deoxycortisol, androstenedione, corticosterone, 11-deoxycorticosterone, testosterone, and progesterone) from a spot,[1] and small-volume steroid ratios correlate closely with plasma and whole blood ($r > 0.93$).[5] Clinicore's panel extends this multiplex approach across five domains from one ~600 µL capillary collection: adrenal steroids, sex steroids with binding protein, gonadotropins and pituitary hormones, a metabolic marker, and vitamin D (Figure 1).

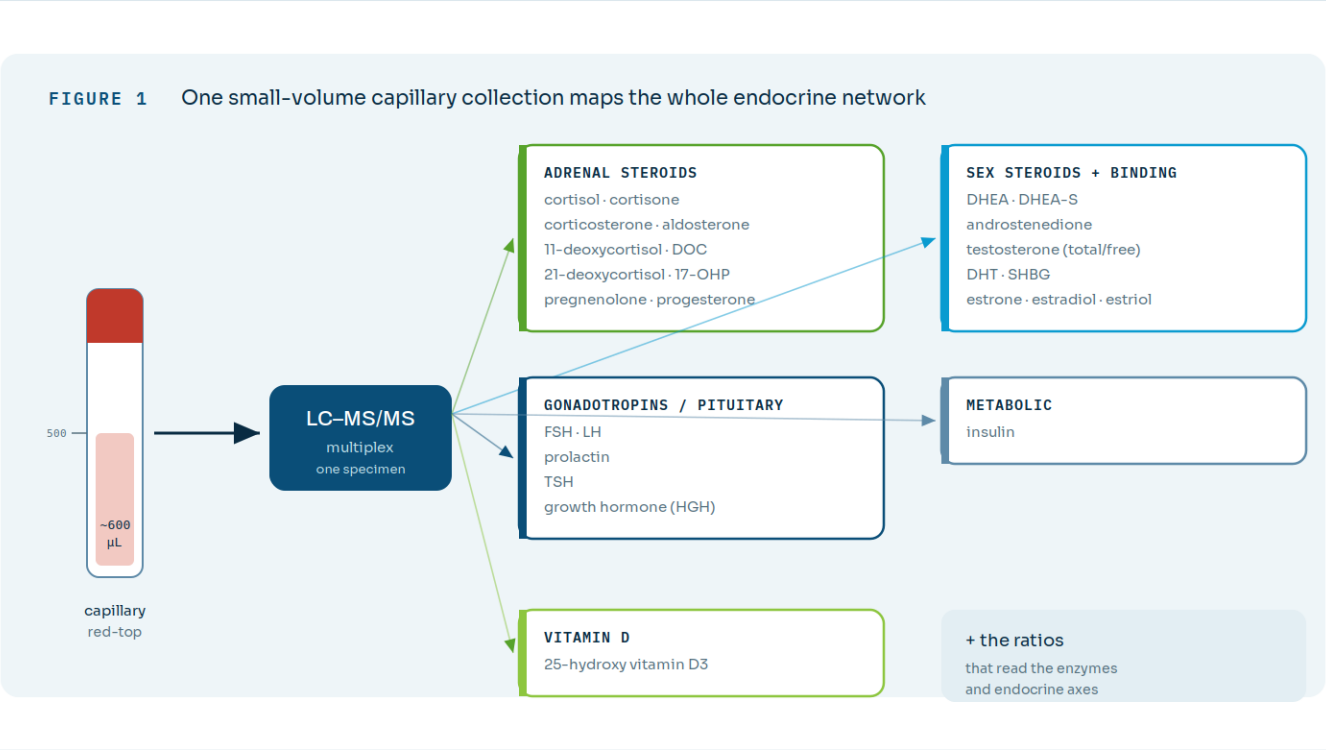

Figure 1. One small-volume capillary collection, processed by multiplexed LC–MS/MS, maps five endocrine domains at once — adrenal steroids, sex steroids and binding protein, gonadotropins and pituitary hormones, a metabolic marker, and vitamin D — plus the ratios computed from them.

3 The steroidogenic tree

Most of the panel's steroids are nodes on a single biosynthetic tree that runs from cholesterol through pregnenolone and progesterone into four branches: mineralocorticoids (deoxycorticosterone, corticosterone, aldosterone), glucocorticoids (11-deoxycortisol, cortisol, and its inactive partner cortisone), androgens (DHEA, androstenedione, testosterone, DHT), and estrogens (estrone, estradiol, estriol), with sex-hormone-binding globulin setting bioavailability (Figure 2). Because the panel measures precursors and products together, the ratio of a product to its precursor reports the activity of the enzyme between them — the principle behind reading 21-hydroxylase from 17-hydroxyprogesterone in congenital adrenal hyperplasia, and behind product-to-precursor ratios that localize steroidogenic

lesions.[63] A single multiplexed panel therefore does more than list hormones; it exposes the enzymatic structure of the pathway.

FIGURE 2 One steroidogenic tree — and the panel measures nearly every node

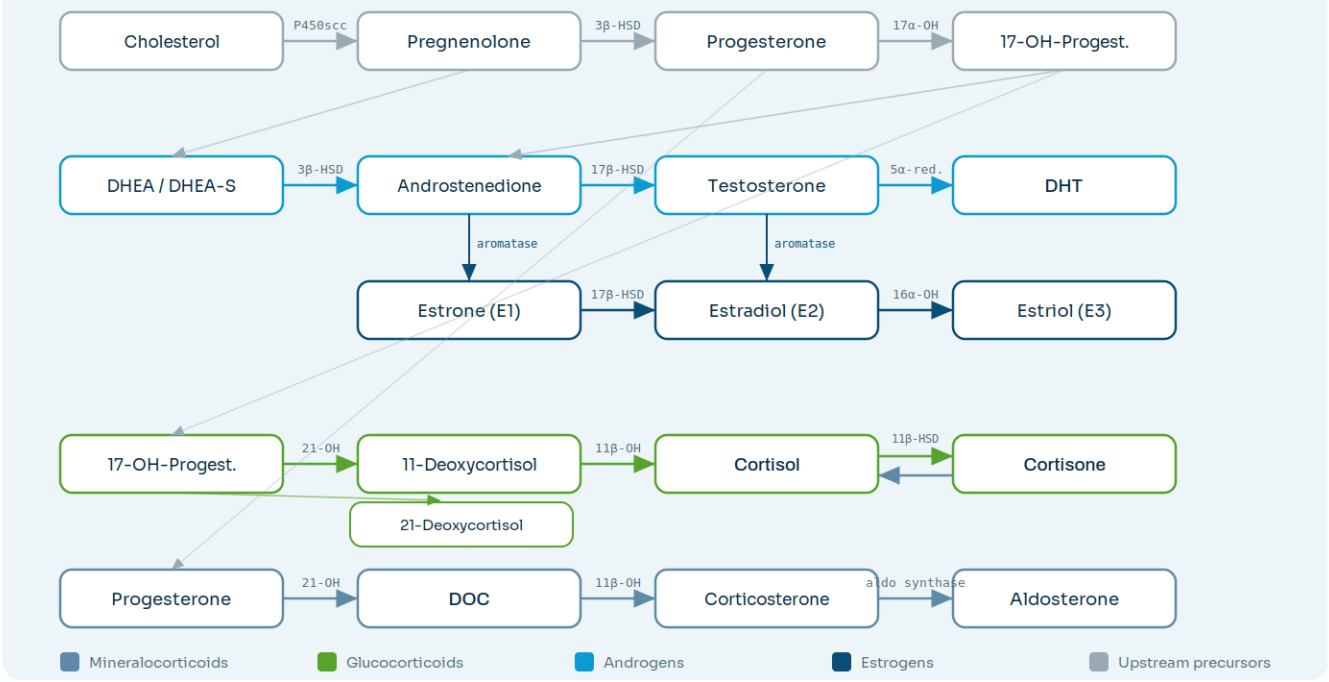

Figure 2. The steroidogenic tree. From cholesterol, four branches produce mineralocorticoids, glucocorticoids, androgens, and estrogens; nearly every colored node is measured on the panel. Product-to-precursor ratios across each enzymatic step read the enzyme between them.

4 Reading the panel, axis by axis

4.1 Adrenal / corticosteroid pathway

Cortisol, cortisone, corticosterone, aldosterone, 11-deoxycorticosterone, 11-deoxycortisol, 21-deoxycortisol, 17-hydroxyprogesterone, pregnenolone, and progesterone together cover the adrenal tree. The cortisol-to-cortisone ratio indexes 11β-HSD2 activity;[7] 17-hydroxyprogesterone (with 21-deoxycortisol) flags 21-hydroxylase deficiency;[6] and multi-steroid profiling has been shown to subtype adrenal disorders by pattern rather than by any single value.[4]

4.2 Sex steroids and binding

DHEA, DHEA-S, androstenedione, testosterone, DHT, estrone, estradiol, estriol, and SHBG map the sex-steroid pathway. The panel supports the diagnostic ratios developed across this series — testosterone:androstenedione (17β-HSD), DHT:testosterone (5α-reductase), and estradiol:estrone (aromatization and menopausal status) — and, because it includes SHBG, calculated free testosterone and the free androgen index, which reflect the bioavailable fraction more accurately than total testosterone alone.[8] Accurate low-level estrogen and androgen measurement requires the sensitivity of LC–MS/MS.[11][10]

4.3 Gonadotropins and pituitary

FSH, LH, prolactin, TSH, and growth hormone place the peripheral steroids in their pituitary context. The LH-to-FSH ratio is a recognized feature of polycystic ovary syndrome, where an elevated ratio accompanies hyperandrogenism and metabolic change.[9]

4.4 Metabolic integration

Insulin ties the panel to the metabolic dimension of endocrine disease. In PCOS in particular, hyperinsulinemia accompanies the hormonal picture, and the guideline-endorsed androgen work-up is most reliable by LC–MS/MS.[9][10]

4.5 Vitamin D status

25-hydroxyvitamin D is the accepted marker of vitamin-D status and the preferred test recommended by scientific bodies, rounding out the panel's coverage of the endocrine–metabolic–bone axis.[12]

5 The ratios that matter

Because the panel measures the pathway rather than isolated points, a set of clinically meaningful ratios can be computed from a single collection. Each reads either an enzyme (a product-to-precursor step) or a physiologic balance (bioavailability, an axis, or an anabolic–catabolic state). Table 1 consolidates the ratios developed across this review series and the additional ones the full panel makes possible.

Table 1. Clinically useful ratios computable from a single small-volume panel.

Ratio	Reads	Typical context
Cortisol : cortisone	11β-HSD2 activity	Mineralocorticoid hypertension; free-cortisol status
17-OHP (± 21-deoxycortisol)	21-hydroxylase (CYP21A2)	Congenital adrenal hyperplasia; androgen excess
11-deoxycortisol : cortisol	11β-hydroxylase (CYP11B1)	CAH; mineralocorticoid hypertension
Corticosterone → aldosterone	Aldosterone synthase (CYP11B2)	Mineralocorticoid pathway
DHEA : DHEA-S	Active : reservoir	Adrenal androgen dynamics
Testosterone : androstenedione	17β-HSD activity	Androgen production
DHT : testosterone	5α-reductase activity	Peripheral androgen activation
Estradiol : estrone	Aromatization / menopause / route	Estrogen status
Free testosterone / FAI (T : SHBG)	Bioavailable androgen	Hypogonadism; hirsutism / PCOS
Testosterone : cortisol	Anabolic : catabolic balance	Training strain and recovery
Cortisol : DHEA-S	HPA / adrenal balance	Stress; adrenal reserve
LH : FSH	Gonadotropic axis	PCOS; gonadal failure

6 Why one small-volume collection changes the picture

Three properties compound. First, breadth: measuring the network together makes the ratios above available from one specimen, rather than reconstructing them across separate orders drawn on different days. Second, specificity and sensitivity: LC–MS/MS resolves structurally similar steroids that cross-react on immunoassay and remains accurate at the low concentrations seen in women, children, and suppressed states, where immunoassays are biased.[2,11] Third, access: microsampling has been shown to deliver multi-steroid panels and their ratios from dried spots and small volumes with plasma-equivalent results,[15] which lowers the barrier to testing and makes serial monitoring practical. Together, these turn a single small draw into a repeatable, network-level readout.

7 Analytical and practical considerations

Several points govern correct use. Free testosterone is best derived by calculation from total testosterone and SHBG, a validated and practical index of the bioavailable fraction.[8] Steroid results are interpreted against standardized collection conditions, and — as with any endocrine testing — reference intervals and cut-offs are laboratory- and method-specific. The panel is broad but not exhaustive: the aldosterone-to-renin ratio, for example, requires a separate renin measurement, and dynamic testing remains necessary where physiology demands it. Within these bounds, a single multiplexed collection provides an unusually complete first-line endocrine assessment.

8 Conclusions

The endocrine system is a network, and it is most informative read as one. Multiplexed LC–MS/MS and small-volume microsampling make it possible to map adrenal steroidogenesis, the sex-steroid pathway, the gonadotropic and pituitary axis, a metabolic marker, and vitamin D from a single ~600 µL capillary collection — and, because precursors and products are measured together, to compute the ratios that read the enzymes and the axes. One small draw, the whole endocrine map.

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About this review. Educational scientific review prepared by the Clinicore Clinical Science Team (CLIA ID 19D2139173; Laboratory Director A. Schmidt, MD). The primary literature cited was identified via PubMed; source DOIs are listed above. This document summarizes published physiology and analytical considerations for healthcare professionals; it is not a clinical practice guideline and is not a substitute for individualized medical judgment. The panel composition shown reflects a laboratory test menu; available analytes, reference intervals, calculated indices, and methods are laboratory-specific and should be confirmed against the current Clinicore directory of services. Some applications (for example, the aldosterone-to-renin ratio) require additional analytes not included in this steroid/immunoassay panel.