# The Science of Dried Blood Spot Testing

Dried blood spot (DBS) is an important part of the minimally-invasive hormone testing that is the hallmark of ZRT Laboratory. Blood spot testing was originally developed in the 1960s out of a need to screen newborns for phenylketonuria (PKU), since a simple heelstick is more practical than a conventional blood draw in young infants. Later this was broadened to include tests for congenital hypothyroidism[1-4]. Today neonatal screening for PKU and thyroid deficiencies using DBS tests is a routine procedure, and assays for a wide range of other analytes in DBS have been successfully developed[5-15]. The simplicity of sample collection, stability of samples in storage and transport, and excellent correlation of blood spot assays with serum tests, have made it an ideal method for epidemiological and field research studies for a variety of health conditions in both children and adults[16].

## Steroid Hormone Testing in Dried Blood Spot

The ability to measure accurately levels of steroid hormones in DBS[17-21] has important implications for reproductive endocrinology, and also allows effective monitoring of hormone replacement therapy. This is of particular note for sublingual hormone users, for whom saliva testing is not optimal. Hormones held in the mouth as a troche or sublingual drops concentrate locally within the oral mucosa, which results in a higher local concentration in the saliva. This can result in "false high" salivary test results for up to 36 hours, depending on many factors responsible for clearing the locally concentrated hormone from the oral mucosa, including the ability to produce saliva, frequency and types of meals and beverages consumed, and toothbrushing. The blood spot assay circumvents this problem of "false-high" test results seen in saliva of sublingual hormone users because the capillary blood is taken from a site distal to the oral mucosa, the finger.

DBS testing has distinct advantages over conventional serum testing for monitoring topical hormone supplementation. Levels of steroid hormones produced endogenously are remarkably similar in venipuncture serum and finger stick capillary blood spots[21]. However, when hormones are delivered topically (transdermally, sublingually, or vaginally), capillary blood spot levels can be much higher than serum levels (ZRT internal data). Animal studies investigating tissue uptake of topically

## Available Tests

**Female Blood Profile I** –
E3,E2, E1, Pg, T, SHBG, DS, C

**Female Blood Profile II** –
E2, Pg, T, SHBG, DS, C, TSH, fT3, fT4, TPOab

**Male Blood Profile I** –
E2, E1, Pg, T, PSA, SHBG, DS, C

**Male Blood Profile II** –
E2, T, PSA, SHBG, DS, C, TSH, fT3, fT4, TPOab

**Hormone Trio** – E2, Pg, T

**Essential Thyroid Profile** –
fT3, fT4, TPOab & TSH

**Elite Thyroid Profile** –
fT3, fT4, TPOab, TSH, Total T4, Tgbn

**Toxic & Essential Elements - Blood** –
Hg, Cd, Pb, Zn, Cu, Se & Mg

**CardioMetabolic Profile** – Insulin, hsCRP, HbA1c, TG, CH, LDL, HDL, VLDL

**Vitamin D Profile** –
25-OH D2, 25-OH D3, Total

Blood spot is ideal for measuring hormones and other analytes such as insulin, blood lipids, Vitamin D, thyroid hormones, and elements like lead and magnesium. It offers distinct advantages over serum because it eliminates the need for a blood draw – saving patients time and money.


# Hormone Testing

## Minimally-invasive home test kit

delivered hormones have shown a striking discrepancy; high tissue hormone levels and much lower serum levels[22]. Research shows that a physiological dose of 20-40 ng/mL progesterone raises the tissue levels of progesterone to a very high luteal phase level (> 20 ng/g tissue)[22,23]. However, under these same conditions, venipuncture serum progesterone levels only increase marginally to sub-luteal levels (1-3 ng/mL). The same is seen with saliva versus serum levels, with much higher hormone levels seen in saliva[24]. We have recently published a clinical study showing saliva levels of progesterone increased 10-fold while capillary blood spot levels increased 100-fold compared to levels in venous whole blood and venous serum following application of 80 mg progesterone cream or gel[25]. This has led us to conclude that when hormones are delivered through the skin or oral or vaginal mucosa, conventional serum hormone tests grossly underestimate hormone delivery to tissues. In contrast, hormone levels in saliva or capillary blood spot better represent tissue hormone uptake. Using only serum test results to monitor topical progesterone supplementation has led to confusion and can result in over-dosing in an attempt to achieve physiological luteal levels of progesterone.

### Other Tests in Dried Blood Spot

In addition to the sex hormones (estradiol, progesterone, DHEA-S, and testosterone), dried blood spot testing is also offered for: morning cortisol, sex hormone binding globulin (SHBG), prostate-specific antigen (PSA), LH, FSH, vitamin D, IGF-1, thyroid testing (TSH, free T3, free T4, total T4, TPO antibodies, and thyroglobulin), cardiometabolic risk markers (fasting insulin, hs-CRP, HbA1c, triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, and VLDL cholesterol), and toxic and nutritional elements (cadmium, mercury, lead, zinc, copper, selenium, and magnesium). For more information on these tests please refer to the Provider Data Sheets for the profiles in which they are included.

### Sample Collection

Collection of the blood spots is a relatively simple and nearly painless procedure that can be done at home or by the health care practitioner. A simple nick of the finger followed by placing blood drops on a filter card is all that is needed. The kit contains easy step-by-step instructions, skin cleansing wipes, two lancets, a filter paper on which the blood drops are collected, and a band-aid. The dry blood spot sample requires no special handling and is returned, together with a requisition form completed by the patient indicating any current hormone therapy and symptoms, to the laboratory for analysis in a pre-paid return package. Blood spot samples are collected in the morning before eating or drinking. Topical hormone users should use their hormones daily as usual but avoid applying the hormones with the hands for several days prior to collection.

### Advantages

- Convenient for both patient and health care practitioner
- No phlebotomist, special preparation such as centrifugation of the blood, or special packaging and shipment required, therefore less expensive and more convenient than conventional blood draws
- Simple and convenient collection of blood at home allows for flexibility of testing at the right time of day or month or following hormone therapy
- Hormones and other analytes stable in dried blood spot at room temperature for weeks, allowing for greater latitude in collection and shipping
- Infectious agents such as HIV are inactivated by drying the blood thus allowing for safer transport and lab testing of the blood sample
- Familiarity of hormone test levels: ranges for hormones in dried blood spots nearly identical to ranges for conventional serum tests
- Levels of elements in dried blood spot correspond to those seen in whole blood tests

## Clinical Utility

Dried blood spot testing can help providers:

- Identify hormonal deficiencies or imbalances associated with aging and disease, thyroid dysfunction and symptoms of menopause and andropause
- Link clinical symptoms to specific hormone imbalances identified by the test
- Restore hormonal balance and patient quality of life using test results as a rational basis for treatment
- Monitor patient hormone levels for individualized, physiologic dosing of hormone supplementation
- Track patient progress with comparative history reports provided with follow-up testing

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