

# About Blood Spot Testing



## The Science of Blood Spot Testing

Blood spot and saliva are both now used for 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<sup>1-4</sup>. Today neonatal screening for PKU and thyroid deficiencies using blood spot tests is a routine procedure, and assays for a wide range of other analytes in blood spot have been successfully developed<sup>5-15</sup>. 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<sup>16</sup>.

The ability to measure accurately levels of steroid hormones in blood spots<sup>17-21</sup> 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.

Blood spot 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<sup>21</sup>. However, when hormones are delivered topically (transdermally, sublingually, or vaginally), capillary blood spot levels can be much higher than serum levels (ZRT internal data). Studies investigating tissue uptake of topically delivered hormones have also shown a striking discrepancy; high tissue hormone levels and much lower serum levels<sup>22</sup>. For example, topically delivered progesterone at a commonly used physiological dose results in high luteal phase levels of progesterone in capillary blood spots (20-40 ng/ml) and research shows that the same physiological dosing raises the tissue levels of progesterone to a very high luteal phase level (> 20 ng/g tissue)<sup>22,23</sup>. 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<sup>24</sup>. This has led to the hypothesis 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.



## Blood Spot 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 blood spots nearly identical to ranges for conventional serum tests

## Clinical Utility

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

## Customer Support

- ZRT's evaluation report includes test results, details of supplements and current symptoms reported by the patient, and ZRT analysis
- The report is returned to the patient or ordering healthcare provider in 5 – 7 business days and is also available via secure internet access
- ZRT staff physicians are available for enquiries without appointment, 8:00 a.m. to 5:00 p.m. weekdays

## REFERENCES

1. Umehashi H, Hayashida T, Otomo S, Fujimoto S, Matsuda I. [The evaluation of measurement of TSH in blood spot on dried filter paper using enzymeimmunoassay] *Horumon To Rinsho* 1983; 31:169-72.
2. Hearn TL, Hannon WH. Interlaboratory surveys of the quantitation of thyroxin and thyrotropin (thyroid stimulating hormone) in dried blood spot specimens. *Clin Chem* 1982; 28:2022-5.
3. Fujimoto S, Kodama M, Suga H, Namikawa T, Namikawa T. [Measurement of antithyroid antibodies using the dried blood spot (author's transl)] *Horumon To Rinsho* 1980; 28:1201-3.
4. Irie M, Enomoto K, Naruse H. Measurement of thyroid-stimulating hormone in dried blood spot. *Lancet* 1975; 2:1233-4.
5. Mitchell ML, Hermos RJ, Moses AC. Comment on dried blood spot assay of IGF-I and IGFBP-3. *J Clin Endocrinol Metab* 1999; 84:822-3.
6. Cook JD, Flowers CH, Skikne BS. An assessment of dried blood-spot technology for identifying iron deficiency. *Blood* 1998; 92:1807-13.
7. Parker DR, Bargiata A, Cowan FJ, Corall RJ. Suspected hypoglycaemia in out patient practice: accuracy of dried blood spot analysis. *Clin Endocrinol (Oxf)* 1997; 47:679-83.
8. Rattenbury JM, Herber SM, Price KJ. Dried blood spot glucose measurement in hyperinsulinaemic hypoglycaemia. *Clin Biochem* 1989; 22:301-3.
9. Beesley R, al Serouri A, Filteau SM. Measurement of C-reactive protein in dried blood spots on filter paper. *Trans R Soc Trop Med Hyg* 2000; 94:348-349.
10. Cordon SM, Elborn JS, Hiller EJ, Shale DJ. C-reactive protein measured in dried blood spots from patients with cystic fibrosis. *J Immunol Methods* 1991; 143:69-72.
11. McDade TW, Burhop J, Dohnal J. High sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. *Clin Chem* 2004; 50:652-4.
12. Quraishi R, Lakshmy R, Prabhakaran D, Mukhopadhyay AK, Jaikhani B. Use of filter paper stored dried blood for measurement of triglycerides. *Lipids Health Dis* 2006; 5:20.
13. Butter NL, Hattersley AT, Clark PM. Development of a blood spot assay for insulin. *Clin Chim Acta*. 2001; 310:141-50.
14. Shields BM, Knight B, Shakespeare L, Babrah J, Powell RJ, Clark PM, Hattersley AT. Determinants of insulin concentrations in healthy 1-week-old babies in the community: applications of a bloodspot assay. *Early Hum Dev*. 2006; 82:143-8.
15. Dowlati B, Dunhardt PA, Smith MM, Shaheb S, Stuart CA. Quantification of insulin in dried blood spots. *J Lab Clin Med* 1998; 131:370-4.
16. Parker SP, Cubitt WD. The use of the dried blood spot sample in epidemiological studies. *J Clin Pathol* 1999; 52:633-9.
17. Worthman CM, Stallings JF. Hormone measures in finger-prick blood spot samples: new field methods for reproductive endocrinology. *Am J Phys Anthropol* 1997; 104:1-21.
18. Worthman CM, Stallings JF. Measurement of gonadotropins in dried blood spots. *Clin Chem* 1994; 40:448-53.
19. Shirtcliff EA, Reavis R, Overman WH, Granger DA. Measurement of gonadal hormones in dried blood spots versus serum: verification of menstrual cycle phase. *Horm Behav* 2001; 39:258-66.
20. Shirtcliff EA, Granger DA, Schwartz EB, Curran MJ, Booth A, Overman WH. Assessing estradiol in biobehavioral studies using saliva and blood spots: simple radioimmunoassay protocols, reliability, and comparative validity. *Horm Behav* 2000; 38:137-47.
21. Edelman A, Stouffer R, Zava DT, Jensen JT. A comparison of blood spot vs. plasma analysis of gonadotropin and ovarian steroid hormone levels in reproductive-age women. *Fertil Steril* 2007; 88:1404-7.
22. Waddell BJ, O'Leary PC. Distribution and metabolism of topically applied progesterone in a rat model. *J Steroid Biochem Mol Biol* 2002; 80:449-55.
23. Chang KJ, Lee TT, Linares-Cruz G, Fournier S, de Lignières B. Influences of percutaneous administration of estradiol and progesterone on human breast epithelial cell cycle in vivo. *Fertil Steril*. 1995; 63:785-91.
24. Stanczyk FZ, Paulson RJ, Roy S. Percutaneous administration of progesterone: blood levels and endometrial protection. *Menopause* 2005; 12:232-7.